Genetics of Immunoresponsiveness to Natural Antigens in the Mouse

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I. Introduction

The complex and potent system of mammalian immunity has been produced by natural selection, through the progressive improvement of the less efficient mechanisms of more primitive animals. The teleonomic function of the immunity system that has directed its evolution, is the self-protection against invasion by viruses, bacteria, parasites (anti-infection immunity) and possibly by transformed malignant cells (anti-tumour immunity).

The phylogenetic evolution of the immunity system has therefore been guided by the need to adapt the host defence against diversified and increasingly sophisticated forms of aggression resulting from the host—parasite—environment equilibrium.

The most primitive type of immunity was conferred by phagocytic cells endowed with the ability to engulf and digest invading micro-organisms. This first step of immunity is essentially non-specific and lacks of memory. The second step in evolution was the elaboration of cell-mediated immunity characterized by unrefined specificity. The final step was the acquired ability to synthesize and release highly stereospecific molecules: the antibodies. The cell-mediated and humoral immunity retain memory and therefore take advantage of the immunological history characterizing each individual. Vaccination is based on this immunological memory.

In mammals these three immunity functions coexist and are performed respectively by macrophages, T lymphocytes and B lymphocytes. Each of these functions is specialized in the protection against some infections and scarcely effective or completely inefficient against others. Consequently infections may be classified into three principal types corresponding to the protective efficiency of one or the other of the three immunity functions. This is, of course, a schematic description since two immunity functions may cooperate in the defence against a given infection; nevertheless one of the three parameters is the determinant factor of the resistance.

Macrophages, T lymphocytes and B lymphocytes coexist in the peripheral lymphoid tissue and interact very closely in the induction and regulation of

the immune response either by cell surface products (receptors) or by released molecules (T factors and antibodies).

The most fundamental finding resulting from our investigations is the demonstration that these three cell types, in spite of their close functional integration in the immunoregulatory network, are distinct protagonists of immunity submitted to separate polygenic control. The resulting polymorphic regulation of immunoresponsiveness is the fundamental characteristic of the immunity system strategy ensuring the best possible multidirectional protection at the level of a genetically heterogeneous population.

In fact, this genetic arrangement provides a reasonably good resistance against all types of infection for most individuals grouped around the modal phenotypes of the three immunity parameters. As a result the bulk of the population is efficiently protected against mild endemic infections, whereas only a fraction of individuals will cope efficiently with every severe epidemic and so ensure the survival of the population (see Chapter VI).

This theory of polyvalent protective efficiency of the immunity system is based on the following fundamental findings demonstrated in Selection I: 1. The independent genetic control of humoral and cell mediated immunity (Chapter V.E); and 2. the inverse relation between genetic regulation of antibody responsiveness and macrophage activity (Chapter V.B).

Modern knowledge of the genetics of immunoresponsiveness proceeds from two different experimental approaches:

1. Production by selective breeding of high and low immune responder lines of mice that are homozygous at the loci regulating immunoresponsiveness to natural polydeterminant immunogens, as described in this review (polygenic regulation).

2. Demonstration, in inbred lines of animals, of the monogenic control operated by specific immunity response genes (Ir genes) on the response to one or a few epitopes.

Two types of specific Ir genes have been described: Ir genes controlling the immunity response that are often linked with the major histocompatibility complex (MHC), and Ir genes linked with immunoglobulin structure genes operating on the fine conformation of antibody molecules, affecting specificity, homogeneity, affinity and idiotypic antigenicity.

Specific Ir genes determine responsiveness to antigens of limited heterogeneity such as synthetic polypeptides, iso-antigens or complex antigens administered at threshold doses, where only the most potent determinant of the molecule is immunogenic (*McDevitt* and *Benacerraf,* 1969; *Sela,* 1972; *Mozes* and *Shearer,* 1972; *Benacerraf* and *Dorf,* 1974).

The principal function of specific Ir genes is regulation of interaction and differentiation of the cells participating in immunity responses (*Katz* and *Benacerraf,* 1976; *Rosenthal,* 1978).

In view of the theory formulated above, two questions may be raised: What is the selective value of specific Ir genes and what are the relations between specific Ir genes and the essentially non-specific polygenic regulation of immunoresponsiveness described in this review?

The results obtained in Selection I (Chapter III.A) demonstrate that among

the group of about ten loci controlling antibody responses, one gene is H-2 linked and another is linked with the Immunoglobulin allotype. These two genes therefore have some characteristics of the Ir genes. They account for only about 20% of the total phenotypic effect produced by the group of about ten loci regulating immunoresponsiveness to complex immunogens (Chapter III.A.3).

The selective value of specific Ir genes cannot result from the control of the immunity response to synthetic polypeptide antigens or iso-antigens in a natural environment. It could result from the control of immunity response to threshold doses of natural antigens since infections are supposed to be produced by small inoculum of pathogenic micro-organisms, mimicking threshold antigen doses.

The experimental results obtained in Selection I do not confirm this possibility. In fact, we have demonstrated that antibody response to an optimal dose of Salmonella typhimurium is of polygenic character, but at a threshold immunizing dose the response is controlled by two loci, one of which is H-2 linked (*Sant'Anna* and *Bouthillier*, unpublished results). The resistance to infection produced by a minimal inoculum of living Salmonella typhimurium is much stronger in low than in high immune responder mice (*Biozzi* et al., 1978). In fact the resistance to this type of infection is due to macrophage activity (Chapter VI.A).

In other types of infection such as by pneumococci, the antibody response to bacterial polysaccharide has a protective effect. The antibody response controlled by specific Ir genes could have a selective value in the defence against this type of infection. The results of *Howard* et al. (1972) and *Baker* et al. (1976) do not support this hypothesis, since they demonstrate that antibody response to Pneumococcus polysaccharide is a polygenic trait unlinked with either H-2 locus or Immunoglobulin allotype.

Immunoglobulin allotype linked and H-2 linked Ir genes analogous to those demonstrated in Selection I (Chapter III.A.3) may have a limited selective value as participants in the total polygenic regulation of immunity responsiveness to the antigens of bacteria and parasites studied in Chapter VI.

The selective advantage conferred by monogenic control of the immune response operated by specific Ir genes is presently not clearly understood. There is no definite evidence of MHC-linked resistance to bacterial infections, whereas the MHC may participate in anti-viral or anti-tumour immunity (*McDevitt* et al., 1974; *Zinkernagel* and *Doherty*, 1975; *Klein*, 1975; *Meruelo* et al., 1977). Ir genes could also intervene in the occurrence of auto immune diseases.

The original method used in the study of quantitative genetic regulation of immunoresponsiveness reported in this review is the production, by selective breeding, of high or low responder lines of mice to natural multideterminant immunogens administered at optimal doses. Five selective breedings are described. The first and the most extensively studied is Selection I. Selections I and II are maintained in the Department of Immunogenetics at the Institut Curie in Paris, France. Selections III and IV were carried out in the Department of Immunology of the Instituto Biologico, São Paulo, Brazil. Selection V was performed in the Department of Immunology of the Escola Paulista de Medicina, São Paulo, Brazil.

II. Methods of Selective Breeding and Genetic Analysis

The results of five selection experiments for quantitative antibody responsiveness to various antigens are described in this review article. The different antigens used were sheep erythrocytes (SE) and pigeon erythrocytes (PE) (Selection I); SE only (Selection II); flagellar (f) and somatic (s) antigens of Salmonellae (Selections III and IV respectively); bovine serum albumin (BSA) and rabbit gamma globulin (RGG) (Selection V).

The characteristics of each Selection will be described later. First, we summarize the methods of selective breeding common to the five Selections and the calculations used to analyze the results.

A. Selective Breeding

The individual antibody response was established after immunization with an optimal dose of antigen i.e. the dose inducing the highest serum antibody level in the immunization schedule chosen. The response was measured during the plateau of maximal antibody level. These experimental conditions were established in preliminary experiments in random bred mice.

The phenotypic character selected was therefore "maximal antibody response produced by an optimal immunization".

The serum antibody titre was measured by direct hemagglutination in Selections I and II; by direct flagellar agglutination in Selection III; by direct somatic agglutination in Selection IV; and by passive hemagglutination in Selection V. The technical details have been described in the corresponding references. The agglutinin titre was measured as the highest doubling serum dilution giving a positive agglutination. The results were expressed in terms of either agglutinin titre or log 2 of agglutinin titre. In previous publications relative to Selections I and II, the agglutinin titre was calculated from an 1/10 initial serum dilution while in the publications concerning Selections III, IV, and V it was calculated starting from undiluted serum. *In order to facilitate the comparison of the results obtained in the five Selections, we have expressed, in this article, all the data in terms of agglutinin titre calculated from undiluted serum.*

It should be remembered that the end point of agglutinin assay is always determined in antigen excess, therefore the two factors determining the agglutinin titre i.e. antibody concentration and antibody affinity, participate in the determination of the final score.

The initial populations used to start the selective breeding—the Foundation populations (F_0)—consisted of adult outbred albino mice produced in distinct colonies in order to obtain a large genetic variability. Only Selection II was founded on an outbred population derived from a single breeder.

The two way selective breeding for maximal or minimal antibody response was based on "individual merit" and repeated in each consecutive generation. Assortative mating of the highest responder mice produced the High line (H) and that of the lowest responder mice produced the Low line (L). In each line several pairs were culled at each generation. They were issued from different families to delay, as far as possible, the increase of consanguinity in each line.

Interline crossing and brother—sister mating were excluded during the selective breeding.

As a rule, two non cross-reacting antigens were used in each Selection. They were alternated at each generation in order to avoid the interference of maternally transmitted antibodies on the immunity responsiveness of the offspring. This method was used for the major part of Selection I and for Selections III, IV and V. To evaluate the effect of the alternate use of two non cross-reacting antigens on the response to selection, Selection II was carried out with SE only. The time interval between weaning and immunization was therefore prolonged until elimination, by natural decay, of the maternal antibody. It is evident that the alternation of two antigens speeds up the selective breeding.

B. Genetic Analysis

The results obtained in each Selection demonstrate that the "Quantitative agglutinin response" is submitted to polygenic regulation. It is determined by the cumulative effect of several independent loci occupied by alleles endowed with "good" or "bad" effects on the antibody response. The data must therefore be analyzed by the methods of quantitative genetics.

We here define the terms used in this article and mention the theoretical assumptions on which the calculations are based. For more detailed explanations see *Falconer* (1960), *Cavalli-Sforza* and *Bodmer* (1971), *Bodmer* and *Cavalli-Sforza* (1976).

In genetically heterogeneous as well as in genetically homogeneous populations of mice, the individual responses expressed as log 2 of the agglutinin titre present a normal frequency distribution. The mean titre is then close to the modal titre and the individual titres are symmetrically scattered on both sides. This is therefore an unbiased scale for genetic analysis.

The F_0 populations of the five Selections are genetically heterogeneous. Their phenotypic variance (VP) is thus due to both genetic factors: genetic variance (VG) and to all non-genetic causes of variability resulting from the environment: environmental variance (VE). The VGF_0 is due to the random distribution in the individuals of the "good" and "bad" effect alleles present in the population at an unknown frequency. The assortative mating in successive generations produces a progressive accumulation of the "good" effect alleles in the H line and of the "bad" effect alleles in the L line. The result is a progressive increase in H line, and a progressive decrease in L line, of the mean agglutinin responses, accompanied by a decrease of phenotypic variances of both lines. When the maximal interline separation is reached, which cannot be increased by continuing the selective breeding, the lines are at selection limit. They are then considered homozygous at the level of all the loci controlling the selected character. This assumption will be verified by counter-selection experiments at present under way.

The total response to selection (RT) is the interline difference at selection limit. It is due to the totalled phenotypic effects produced by all the homozygous

loci. The response to selection is also expressed as the mean response per generation (RG) obtained by dividing RT by the number of generations required to reach the selection limit.

Response to selection (R) results from the genetic pressure due to the assortative mating, measured by the selection differential (S). S is the difference between the mean value of the selected parents and that of the generation out of which they have been culled. The number of offspring per selected pair being variable, S was weighted according to litter size. The selection differential per generation (SG) is the mean S value of the generations required to reach the selection limit.

R and S can be calculated either in each line separately or in terms of interline divergence by adding the values of cumulated R or S obtained in corresponding generations of H and L lines.

The mean realized heritability (h^2) calculated by the ratio RG/SG measures the average proportion of the parental deviation which is actually inherited by the progeny. In the absence of dominance, h^2 is a measure of the additive effect of the homozygous loci occupied by "good" or "bad" effect alleles. In the foundation population therefore $VF_0 \times h^2$ is an estimation of the additive variance (VA). An estimate of the number of independent loci controlling the character (n) may be obtained by the formula

$$n = \frac{1}{8} \times \frac{RT^2}{(VF_0 \times h^2)} \tag{1}$$

The meaning and limitations of the calculation of n will be discussed later.

C. Interline Hybrids

These were produced by mating homozygous generations of H and L lines at selection limit. The following crosses were made: $(H \times L) = F_1$; $(F_1 \times F_1) = F_2$; $(F_1 \times H) = BcH$; $(F_1 \times L) = BcL$.

The same number of reciprocal crosses was always made. Since no sex linked difference was observed, the data of both sexes were pooled.

1. Global Dominance

In F_1 hybrids each locus is heterozygous. In the absence of dominance the total additive effect (a) of all the loci of homozygous parental lines is: $a = \frac{1}{2}(\bar{x}H - \bar{x}L)$, then $RT = 2a$.

The dominance of polygenic characters results from the interaction of the unknown dominance effect at the level of each heterozygous locus in F_1 hybrids. It is therefore called global dominance.

The global dominance (d) is: $d = F_1 - \frac{1}{2}(\bar{x}H + \bar{x}L)$.

The proportion of the global dominance effect in relation to the additive effect is measured by the ratio d/a. The value of d/a in the absence of overdominance is between 0 (no dominance) and $+1$ or -1 (complete dominance of high or low responsiveness respectively).

2. Variance Analysis

The environmental variance VE is the phenotypic variance of the genetically homogeneous populations: H and L lines at selection limit and their F_1 hybrids. Therefore

$$VE = \frac{VH + VL + VF_1}{3} \tag{2}$$

The phenotypic variance of the genetically heterogeneous populations F_0, F_2, BcH and BcL is due to both genetic and environmental factors.

The variance of F_2 hybrids (VF_2) is:

$$VF_2 = VA + VD + VE \tag{3}$$

where VA is the additive variance and VD is the dominance variance.

The expected contribution of all the loci to the phenotypic variance of F_2, in the absence of dominance, is the additive variance: $VAF_2 = \frac{1}{2}\sum a^2$ where a is the additive effect. VAF_2 is therefore a measure of the phenotypic difference produced by homozygous loci.

The contribution of the dominance effect, d, to the VF_2 is called dominance variance: $VDF_2 = \frac{1}{4}\sum d^2$.

The variance of each backcross is due to the difference between homozygous and heterozygous loci. VBcs (the addition of VBcH and VBcL) is then:

$$VBcs = VA + 2VD + 2VE \tag{4}$$

From Equation 3 and 4 the values of VA and VD may be directly calculated as follows:

$$VA = 2VF_2 - VBcs \tag{5}$$

$$VD = VBcs - VF_2 - VE \tag{6}$$

There is another way to calculate VA and VD using differences between means rather than variances. If we postulate that individual genes have an equivalent effect or that, if there is a variability it follows a constant pattern then

$$\frac{VD}{VA} = \frac{1}{2}\left(\frac{d}{a}\right)^2 \tag{7}$$

VD may be expressed as a function of VA in Equations 3 or 4, and VA and VD may be calculated from VF_2 or VBcs respectively.

The heritability (h^2) of the character in interline crosses (F_2 or backcrosses) is measured by the ratio of VA on their total phenotypic variance:

$$h^2 = \frac{VA}{VA + VD + VE} \tag{8}$$

If we postulate a theoretical model in which all the relevant loci are completely independent and may be occupied only by two alleles endowed with an equivalent "good" or "bad" effect, then the number of loci (n) controlling the character may be calculated as follows:

$$n = \frac{a^2}{2VA} \tag{9}$$

VA is calculated from the data of interline crosses as previously shown. The calculation of n by Equation 1 and Equation 9 should coincide if no allele were lost by genetic drift during the selective breeding and if the frequency distribution of the relevant alleles in F_0 were close to 0.5.

It should be stressed that the estimate of n is very approximate and must be considered as an order of magnitude rather than a precise figure; nevertheless it permits the comparison of the results obtained in the five Selections. In fact the estimate of n is submitted to the large experimental and sampling errors inherent to the variance calculations and is based on a simplified theoretical model in which many factors are unknown and have to be postulated. The linkages so far demonstrated afford a sound experimental support in favour of the validity of this model for the analysis of the character investigated. In Selection I where ten loci are postulated, two independent linkages were demonstrated, each one accounting for about 10% of RT (Chapter III.A).

III. Results of the Five Selective Breedings

In each Selection the character "antibody response" was submitted to polygenic regulation. The principal results obtained in the five selective breedings are described in this chapter.

A. Selection I for Agglutinin Response to Sheep and Pigeon Erythrocytes

1. Selective Breeding and Genetic Analysis

The Selection was founded on 62 random bred albino mice of both sexes obtained from several commercial breeders. The assortative mating was made by culling at least six pairs per generation in each line. The mean number of mice per generation was 50 ± 15 in H line and 46 ± 15 in L line.

Fertility and fecundity were not appreciably affected by the selective breeding.

The magnitude and the kinetics of agglutinin response were both progressively modified under the effect of selection. Thus, the initial exponential rise of serum agglutinins observed until the 5th day post-immunization in the two lines was followed by a rapid fall in L responders, whilst until the 14th day, an additional rise was observed in H responders (see Fig. 5) (*Biozzi* et al., 1971, 1974).

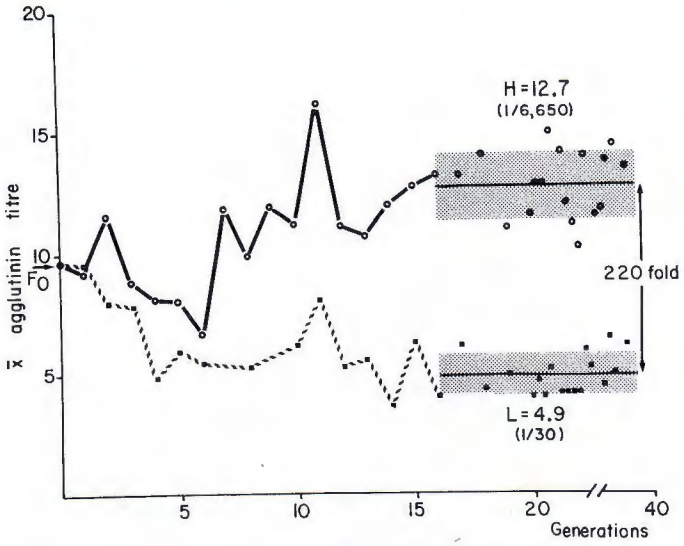

Fig. 1. Selection I. $\bar{x}$ anti SE or PE agglutinin titres (log 2) 14th day post i.v. immunization in successive generations of H and L lines. F_0–F_{16}: Divergence of H and L lines. F_{16}–F_{38}: Total range of interline separation in homozygous generations ± standard deviation

The largest phenotypic difference between H and L lines was found at the 14th day post primary immunization, therefore the results of the selective breeding were calculated according to the 14th day agglutinin titre (*Feingold* et al., 1976). The mean and variance calculations of each generation were made from individual data of both sexes since no constant sex effect was noticed in the generations. A slight female superiority of about 0.5 proved significant only when large populations (>100 mice) were compared.

The results of the selective breeding are represented in Fig. 1.

The agglutinin response of the F_0 population immunized i.v. with 1×10^8 SE was 9.7 ± 1.6. The offspring were weaned when 30 days old and immunized 10 days after weaning. This was repeated for the first six generations. Although the selective breeding produced a significant interline separation, the responsiveness of H line decreased progressively. This depression was due to the effect of a maternally transmitted antibody rather than to an inbreeding depression. In fact a strong response of H line and a large interline difference was observed in groups of F_6 mice immunized with pigeon erythrocytes (PE) that are antigenically unrelated with SE. The 7th generation was therefore immunized i.v. with the optimal dose of 10^8 PE. Afterwards the two immunogens were alternated at each generation in order to avoid the specific effect of passively transmitted maternal antibodies. This antigen alternation speeds up the selective breeding since mice may be immunized shortly after weaning (15 days). After the 13th generation the dose of SE was raised to 5×10^8.

The two lines diverged progressively during the selective breeding until the 16th generation when the maximal interline separation was obtained (selection limit). Afterwards the interline difference remained roughly constant in spite

of the continuation of the selective breeding until F_{38}. These findings may be interpreted as follows: by F_{16} the "good effect" alleles were accumulated in the H line and the "bad effect" alleles in the L line. Both lines could thus be considered as homozygous for the loci controlling the character investigated.

This group of loci produced a very large phenotypic effect since there is a 220-fold difference in agglutinin titre between H and L lines.

The response to selective breeding was asymmetrical. In relation to the F_0 population the immune responsiveness of L line was decreased 28-fold while that of H line was increased only 8-fold in terms of agglutinin titre.

The following considerations on the phenotypic variance only concerns the response to SE since the F_0 population and the interline hybrids analyzed later (Table 1), were immunized with this antigen.

The phenotypic variance of the F_0 population was 2.56. Since this is a genetically heterogeneous population, its variance is due to both genetic and environmental factors: VG and VE respectively. The variance of the successive generations decreased progressively during the selective breeding as their genetic homogeneity increased. It remained fairly constant in the homozygous generations (F_{16}–F_{38}) in which it was produced only by environmental effects (VE). The mean VE of the F_{16}–F_{38} generations immunized with SE was 0.74 in H line and 1.30 in L line. The mean in the two lines was 1.02. From this value it may be calculated that 60% of the VF_0 is attributable to genetic factors ($VGF_0 = 1.54$) and 40% produced by environmental effects.

Environmental factors are responsible for the erratic fluctuations affecting H and L lines alike. Their impact may be reduced if the response to selection is expressed in terms of interline difference, as shown in Fig. 2.

The response to selection R is the difference between the mean agglutinin titre of H and L lines of the same generation. It was cumulated at each generation (cumulated R). The selection differential S is the sum of the S values calculated separately in H and L lines at each generation (cumulated S). The mean values of R and S per generation, RG and SG respectively, were calculated by a least square linear regression from the F_0–F_{16} generations. SG measures the mean value of the selective pressure which produced the mean response to selection, RG.

The results represented in Fig. 2 show that H and L lines diverged progressively during 16 generations of selective breeding. The selection limit was reached in F_{16} where the maximal interline separation was obtained ($RT = 7.8$). From F_{16} onwards the RT value remained constant in spite of the continuation of the selective breeding that produced a steady increase of cumulated S until F_{38}. The dissociation of R from S after selection limit demonstrates that the generations between F_{16} and F_{38} are genetically homogeneous. Their phenotypic variance, entirely due to environmental factors, has no effect on their progeny.

The mean heritability (h^2) realized during the 16 generations of operative genetic selection calculated by a least square linear regression of R/S, was 0.20 ± 0.08. This value represents the mean h^2 of H and L lines since it was calculated from the interline divergence. Because of the asymmetrical response to selection previously mentioned, each line has a distinct h^2. In relation to

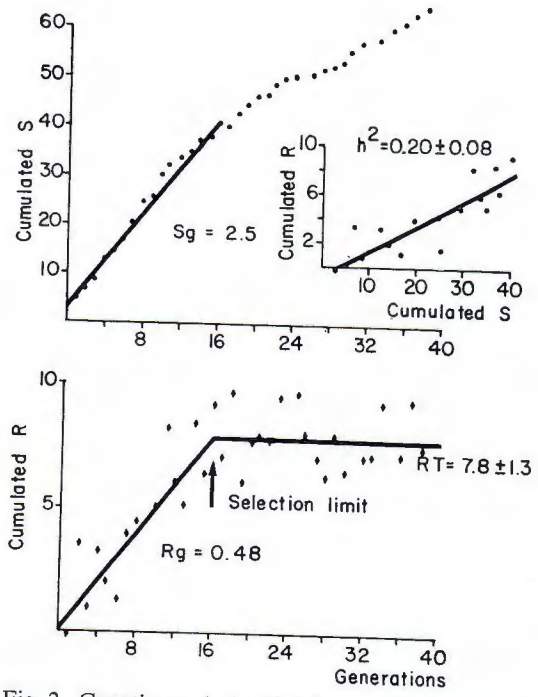

Fig. 2. Genetic analysis of Selection I — cumulated selection differential (S) log 2, in H and L lines — cumulated response to selection (R) log 2, in H and L lines. Calculation of realized heritability (h^2): plot of cumulated R on cumulated S for generations up to selection limit

the F_0 population the RT was 3.0 in H line and 4.8 in L line. The cumulated S in F_{15} was 20.2 and 19.7 in H and L lines respectively. Consequently h^2 was 0.15 in H line and 0.24 in L line. The difference in the h^2 values of H and L lines is mostly due to the incomplete dominance of high responsiveness (Table 1), which limits the response to selection in the direction of the dominance effect.

According to Equation 1 the number of independent loci may be calculated from RT, h^2 and VF_0. The result of this calculation indicates that a group of about 15 independent loci regulates the agglutinin responsiveness to SE. This estimate will be compared with the results obtained in interline hybrids (Table 1).

2. Interline Crosses

The agglutinin response to SE was measured in interline hybrids: F_1, F_2, and in the 2 backcrosses, BcH and BcL.

The following genetic analysis is based on the probable assumption that H and L lines at selection limit are homozygous at the level of all the loci controlling the character investigated.

Table 1. Mean agglutinin titres and variances in homozygous generations of H and L lines and in interline hybrids of Selection I (14th day post-immunization with the optimal dose of 5×10^8 SE)

Line	Number of mice	Log 2 agglutinin titre	
		Mean $\bar{x}$	Variance V
H line F_{16}–F_{36}	472	12.50	0.74
L line F_{16}–F_{36}	497	4.67	1.30
F_1 (H × L)	211	9.63	1.62
F_2 ($F_1 \times F_1$)	363	8.62	1.97
BcH (F_1 × H)	166	11.36	0.99
BcL (F_1 × L)	168	7.59	2.30

a = 3.915 d = 1.045 d/a = 0.27

The results concerning H and L lines were established as the mean values of all the homozygous generations immunized with SE. These data and the results obtained in interline crosses are reported in Table 1 (see also *Biozzi* et al., 1979).

a) Evaluation of the Global Dominance

The data in Table 1 show that the mean response of F_1 hybrids was closer to that of H than to that of L line. Hence there is an incomplete dominance effect of high over low responsiveness (d/a = 0.27). The dominance effect is 27% of the additive deviation, a. In F_1 where each locus is supposed to be heterozygous, the frequency distribution of the alleles in the F_1 population is 0.5. The mean of the F_0 population ($\bar{x}F_0 = 9.7$) was very close to that of F_1, therefore the frequency distribution of the relevant alleles in the F_0 population should also be close to 0.5. As mentioned before (paragraph 1) it is very probable that the asymmetrical response to selection is essentially due to the dominance effect. This conclusion eliminates the other possible causes of the asymmetrical response namely: uneven distribution of relevant alleles in the F_0 population and intervention of a different number of loci in H and L lines.

The mean responses of the other interline crosses F_2, BcH and BcL are also affected by the incomplete dominance of high responsiveness demonstrated in F_1. The mean ratio of d/a measured in F_1, F_2, BcH and BcL is 0.30 ± 0.18.

The environmental variance (VE) measured as the mean of the three genetically homogeneous populations (H, L and F_1) is 1.22 (Equation 2). The partition

Table 2. Comparison of the different estimates of variance components, heritability and number of loci in Selection I

Environmental variance VE Equation 2	Method of calculation of VA and VD	Partition of genetic variance VG		Heritability h^2 Equation 8	Number of loci n Equation 9
		Additive variance VA	Dominance variance VD		
1.22	Ⓐ From VF_2 and VB_{cs} Equations 5 and 6	0.65	0.10	0.33	11.7
	Ⓑ In VF_2 Equations 3 and 7	0.72	0.026	0.36	10.6
	Ⓒ In VBcs Equations 4 and 7	0.79	0.029	0.39	9.7
	Ⓓ In F_0 Equation 1	0.51	0.83	0.20*	14.8

* Mean value of h^2 realized during the selective breeding (Fig. 1)

between the genetic and the environmental origin of the variance is, in F_2: VG=38% and VE=62%; in BcL: VG=47% and VE=53%. Because of the dominance of high responsiveness the variance of BcH is within the range of VE values. These results underline the large impact of environmental factors in the phenotypic variability of interline segregants.

b) Evaluation of the Components of the Phenotypic Variance, Heritability and Number of Relevant Loci

The results of the variance analysis of the agglutinin response in interline hybrids are shown in Table 2. This type of analysis is subject to large sampling and experimental errors. Nevertheless the results are reasonably consistent.

The evaluation of VA and VD made according to the three methods of calculation, A, B and C, indicated in Table 2, gives concordant results. The difference in VA figures is not significant since the sampling error of VA calculated by method A is 0.13. The genetic variance of F_2 and backcrosses is almost entirely due to the additive effect; the contribution of the dominance variance (VD) is 15% according to calculation A and 4% according to calculations B and C. Because of the constancy of the VA estimate, the evaluation of h^2 in interline crosses by the three methods of calculation, A, B and C, gives similar results. The results obtained by method D differ slightly but are consistent with those mentioned above. In fact the VA established by calculation D concerns the F_0 population and the h^2 refers to the h^2 realized during the selective breeding (Fig. 2). The mean value of VA and h^2 obtained by the four estimations shown in Table 2 is: $VA=0.67\pm0.11$, $h^2=0.28\pm0.07$. It is remarkable that the estimate of n calculated from the data obtained during the selective breeding (Equation 1) coincides with that made in interline hybrids (Equation 9).

The final conclusion is that the quantitative agglutinin response to SE is regulated by a group of about ten independent loci. This estimate is confirmed by the results of the two distinct linkages demonstrated in interline segregants.

3. Demonstration of Two Independent Linkages

Two independent linkages have been demonstrated so far for two loci among the group of ten which regulate the immune response in H and L mice.

a) Immunoglobulin Allotype Linked Locus

It has been recognized that H and L mice differ in the Ig heavy chain structure genes (*Biozzi* et al., 1970). The study of agglutinin response to SE in interline F_2 hybrids and in backcrosses demonstrated a positive correlation between the Ig allotype distribution and the agglutinin level (*Lieberman* et al., 1972). The mean agglutinin titre in F_2 hybrids homozygous for the H line Ig allotype was 9.8 whereas it was 8.8 in F_2 hybrids homozygous for the L line Ig allotype ($p < 0.01$). This difference compared with the RT value (7.8) means that the quantitative contribution of the allotype linked locus is 13% of the total phenotypic difference between homozygous H and L lines.

b) H-2 Linked Locus

Experiments of skin graft exchanged between H and L lines and serum lymphocytotoxicity tests demonstrated that the two lines differ at the major histocompatibility locus H-2 (*Liacopoulos-Briot* et al., 1972). The distribution of H and L line H-2 phenotypes in F_2 hybrids and backcrosses was established by a lymphocyte cytotoxicity test in presence of C′ using specific allo antisera.

A positive correlation was found in F_2 hybrids and backcrosses, between the H-2 phenotype and the SE agglutinin response. In F_2 hybrids homozygous at the H-2 locus of H line the SE agglutinin titre was 10.7 and it was 9.3 in F_2 mice homozygous at the L line H-2 locus ($p < 0.001$). The phenotypic effect produced by the H-2 linked locus was 1.4.

This difference is 18% of that separating H and L lines homozygous at the level of all the ten loci regulating SE responsiveness ($RT = 7.8$) (*Stiffel* et al., 1974). In other experiments described in paragraph 4 the contribution of the H-2 linked locus was estimated as 10% of the RT value.

As mentioned above, the quantitative effect of these two identified loci confirms the estimate of the total number of relevant loci since the model used to calculate n postulates that each locus has an equivalent effect of 10% of RT value.

4. Genetic Control of Responsiveness to Threshold Doses of Sheep Erythrocytes

The results described so far concern the response to an optimal dose of SE, i.e., the character used to carry out the selective breeding.

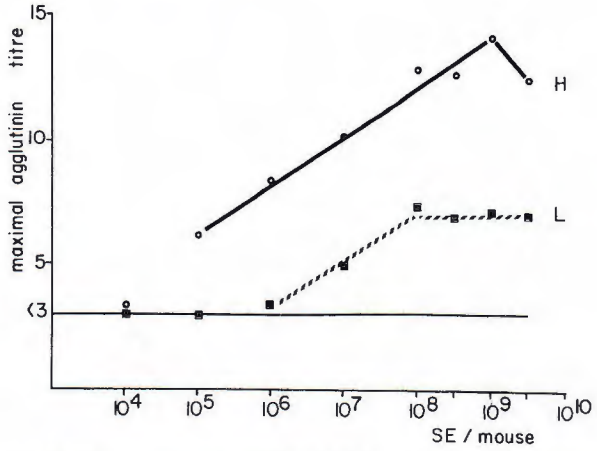

Fig. 3. Dose-response relationship in H and L lines of Selection I: peak agglutinin titres (log 2) in groups of 5–10 mice from each line, immunized i.v. with increasing doses of SE (titres < 3 = background titre)

In other experiments the antibody response was measured in groups of H and L mice immunized with a large range of SE doses, from sub-immunogenic to supra-maximal. The peak agglutinin titres in both lines for each antigen dose are shown in Fig. 3.

In the range of dose-response relationship, the interline difference was constant and independent from the antigen dose (*Biozzi* et al., 1972a). The sensitivity to immunogenic stimulation was very different in the two lines. The threshold dose of antigen required to induce a detectable response was about 100-fold lower in H than in L mice. For the dose of 10^6 SE, H and L mice could be classified as "responders" and "non-responders" respectively, since the titre of natural SE agglutinin is $\leqq 3$.

The inheritance of the threshold character "responsiveness to 10^6 SE" investigated in interline hybrids, F_1, F_2 and both backcrosses is presented in Table 3.

It is evident that the genetic regulation of responsiveness to 10^6 SE is quite different from that described for the optimal dose of SE (Tables 1 and 2). The two fundamental differences concern the dominance and the number of loci involved. At the optimal immunizing dose: 5×10^8 SE, there was an incomplete dominance effect of high response in F_1 hybrids: $d/a = 0.27$ (Table 1). This effect was reversed by decreasing the dose of SE: for the dose of 10^6 SE, $d/a = -0.53$ and for 10^5 SE a complete dominance of low responsiveness was observed.

The number of relevant loci, n, can be evaluated either by the variance analysis as reported in Table 3, or by comparing the distribution of parental phenotypes in segregant interline hybrids with that expected according to Mendelian inheritance (*Stiffel* et al., 1974). Both methods give concordant results which demonstrate that two loci intervene in the control of the antibody response to 10^6 SE.

Table 3. Mean agglutinin titres and variances in H, L and interline hybrids of Selection I (10th day, post-immunization with the threshold dose of 10^6 SE). Resulting genetic parameters

Line	Number of mice	Log 2 agglutinin titre	
		Mean $\bar{x}$	Variance V
H line	40	8.4	2.94
L line	41	3.56	0.29
F_1 (H × L)	31	4.7	1.60
F_2 ($F_1 \times F_1$)	120	5.9	3.77
BcH ($F_1 \times H$)	42	6.60	4.95
BcL ($F_1 \times L$)	43	3.53	0.85

$a = 2.42$ $d = -1.28$ $d/a = -0.53$
$VA = 1.6$ (Equation 5), $n = 1.8$ (Equation 9)

The antibody response to the optimal dose of 5×10^8 SE is controlled by about ten loci (Table 2), one of which is H-2 linked whereas only two loci intervene in the control of the response to the threshold dose of 10^6 SE. It was important to investigate whether one of the 2 loci was H-2 linked. In order to study the quantitative effect of H and L H-2 phenotypes in large groups of mice, the following experiment was designed. F_2 mice homozygous for either H or L phenotypes were mated. They produced two populations referred to as F_3 H/H and F_3 L/L respectively. These mice differ only at the level of the H-2 locus, while the background genes as well as the other genes controlling the immunity regulation are distributed at random as in an F_2 population. Thus the mean difference between the two groups of F_3 H/H and F_3 L/L is an exact measure of the H-2 linked locus effect. These two populations were immunized with either the optimal (5×10^8) or the threshold (10^6) dose of SE. The results are shown in Fig. 4 as individual maximal agglutinin titres.

The difference between the mean responses to 5×10^8 SE in F_3 H/H and F_3 L/L is significant ($p < 0.02$); it is equal to 10% of the interline difference between the two homozygous lines ($RT = 7.8$, Fig. 2). This estimation is consistent with the previously mentioned data obtained in F_2 hybrids differing at the level of the H-2 locus.

A greater difference in agglutinin response was observed between F_3 H/H and F_3 L/L immunized with 10^6 SE. It was evident that almost all the mice homozygous for the H-2 phenotype of H line were "responders" to this antigen dose, as H mice are. On the contrary, in F_3 L/L mice, an important percentage, but not all, were "non-responders", as L mice are. This finding is explained by

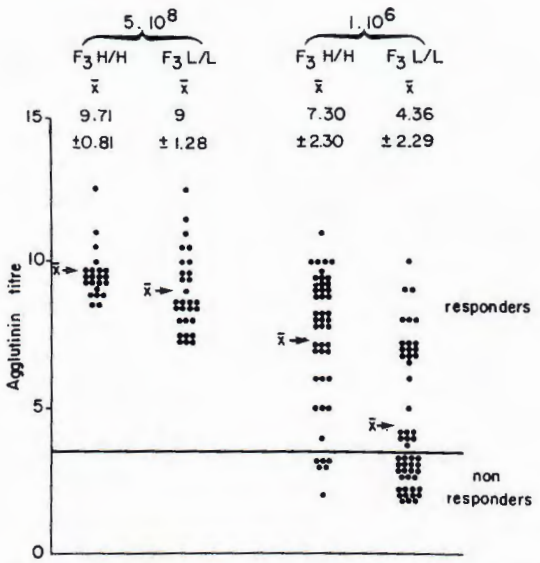

Fig. 4. Individual maximal agglutinin titres (log 2) in F_3 H/H and F_3 L/L mice (having the H-2 phenotype of H and L mice respectively on a F_2 background) immunized i.v. with 5×10^8 or 10^6 SE (mice with titres $\leqq 3$ are classified "non responders" as L mice)

the intervention of the second locus participating in the regulation of responsiveness to the threshold dose of SE. 73% of the total number of F_3 H/H and F_3 L/L mice gave the response expected according to the parental H-2 phenotype. The difference between the mean agglutinin titres in F_3 H/H and F_3 L/L compared with that observed between H and L lines, for the same antigen dose, demonstrated that 61% of the interline difference is due to the H-2 linked locus (*Mouton* et al., to be published).

The quantitative effect of the H-2 linked locus is 10% of the interline difference for the optimal dose of SE and 61% of the interline difference for the threshold dose of SE. Since the regulation is operated by ten loci in the first instance and by two loci in the second, the quantitative contribution of the H-2 linked locus fits with the calculations of the number of loci made by the variance analysis (Tables 2 and 3).

These results deserve the following concluding remarks. The complexity of the genetic regulation of antibody response to SE decreases with the dose of antigen administered. Ten loci operate at the optimal immunizing dose and only two loci at the threshold dose. For both doses an H-2 linked locus is involved. The H-2 linked locus operating at the threshold dose of SE has some characteristics of a specific H-2 linked Ir gene discriminating responders from non-responders to threshold doses of native antigens (*Vaz* et al., 1970, 1971; *Benacerraf* and *McDevitt*, 1972; *Benacerraf*, 1973). Two important differences between our model and the monogenic control operated by Ir genes should be stressed: the intervention of a second locus for the responsiveness to the threshold dose of SE and the opposite dominance effect. In the H-2

linked specific Ir model the high response is dominant in F_1 hybrids whereas in our model the low response is dominant. Another similar example concerning the responsiveness to small doses of hen egg albumin in H and L lines is reported in Chapter IV.A.

5. Kinetics of 19S and 7S Agglutinin Production During Primary and Secondary Responses

Selective breeding was carried out for the agglutinin titre during the primary response. The results reported in Fig. 5 demonstrate that both primary and secondary responses to SE are modified in H and L lines at selection limit (*Biozzi* et al., 1974).

The kinetics of the total agglutinin response after primary immunization was very different in H and in L lines (Fig. 5A). After an initial exponential rise for 4–5 days in both lines the agglutinin level decreased rapidly in L line, whereas it increased and persisted at very high levels in H line. After the second challenge both lines presented a secondary response though the interline difference was smaller than after primary immunization.

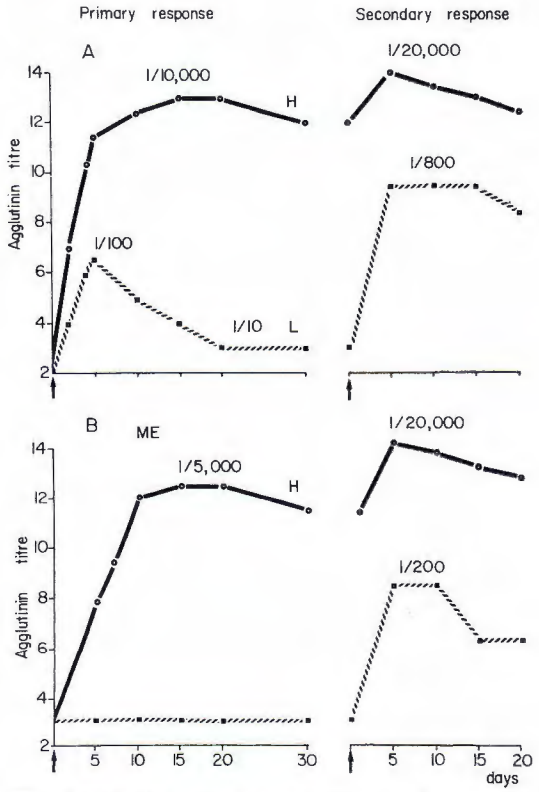

Fig. 5. Kinetics of primary and secondary responses to i.v. immunization with 5×10^8 SE in H and L lines of Selection I. $\bar{x}$ titres (log 2) of total (A) and ME resistant (B) agglutinin in groups of 10–15 mice from each line

These results indicate that both primary and secondary responses are submitted, at least partially, to the same genetic control.

Figure 5B represents the kinetics of primary and secondary agglutinin responses established in samples of immuneserum treated with mercaptoethanol (ME) in order to destroy the 19S agglutinins.

During the primary response H mice produced both 19S and 7S agglutinins with a quantitative preponderance of the latter. L mice on the contrary only produced 19S agglutinins.

During the secondary response only 7S antibodies were synthesized in H line whilst both antibody classes were produced in L line.

These findings indicate that the genes regulating immunoresponsiveness operate on the synthesis of the two major classes of antibody: 19S and 7S.

The effect on the other classes and sub-classes of antibody are described in Chapter V.C.

B. Selection II for Agglutinin Response to Sheep Erythrocytes

1. Selective Breeding and Genetic Analysis

In this Selection all the generations were immunized with the same antigen: SE. The mice were weaned 30 days after birth. The period between weaning and immunization was extended to 60–70 days in order to eliminate the maternal antibodies. The F_0 population consisted of 50 random bred albino mice of both sexes obtained from a single commercial breeder. At least seven pairs per generation were selected in each line. The mean number of mice per generation was 56 ± 13 in H line and 58 ± 14 in L line.

The immunization was given intravenously. The SE dose was 10^8 until the sixth generation and 5×10^8 afterwards.

The kinetics of agglutinin response measured in H and L lines at selection limit was very similar to that of Selection I reported in Fig. 5. As for Selection I, the greatest difference between H and L responders was found 14 days after primary immunization; therefore the 14th day agglutinin titer was considered as the phenotypic measure for the agglutinin responsiveness. As for Selection I a small superiority in the responses of female mice was noticed. The mean of this sex effect calculated in the 22nd generation of H and L lines was 0.6. The means and variances of each generation were calculated from individual data of both sexes (*Feingold* et al., 1976).

The results of the selective breeding are shown in Fig. 6.

The response of the F_0 population was 10.1 ± 1.56. It was almost identical to that of the F_0 population of Selection I, which suggests a similar genetic constitution of the two F_0 populations.

The H and L lines diverged progressively during the first 13 generations. Afterwards, the interline difference remained roughly constant in spite of the continuation of the selective breeding until F_{22}. Selection limit was reached in F_{13}.

The mean response of the generations considered as homozygous for the genes controlling the responsiveness (F_{14}–F_{22}) was 11.6 in H line and 4.9 in

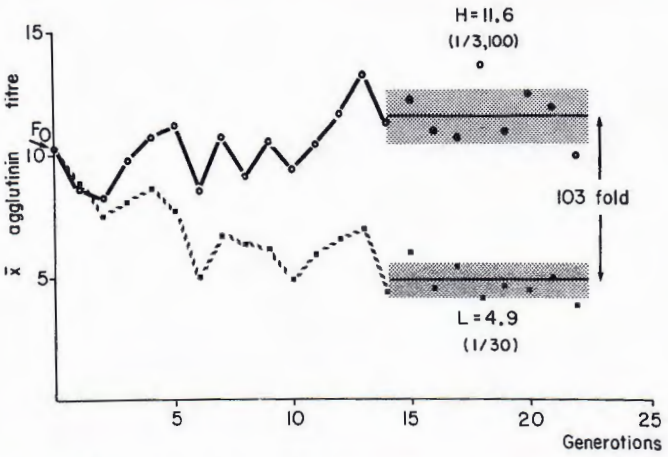

Fig. 6. Selection II. $\bar{x}$ anti SE agglutinin titres (log 2) 14th day post i.v. immunization in successive generations of H and L lines. F_0–F_{14}: Divergence of H and L lines. F_{14}–F_{22}: Total range of interline separation in homozygous generations ± standard deviation

L line. The maximal interline separation was 103-fold in terms of agglutinin titre.

In Selection II also there was an asymmetrical effect of the selective breeding which produced a greater downwards than upwards response to selection in relation to the level of the F_0 population. In fact the responsiveness of L line was decreased 36-fold whereas that of H line was increased only 3-fold.

The environmental variance, VE, calculated as the mean of the homozygous generations (F_{14}–F_{22}) of the two lines was 1.24; the phenotypic variance of the F_0 population VF_0 was 2.43. Therefore 49% of the VF_0 is due to genetic factors ($VGF_0 = 1.19$) and 51% to environmental effects.

The response of F_1 interline hybrids (10.06) (Table 4) was identical to that of the F_0 population, therefore the asymmetry is due to the dominant effect of high responsiveness.

The cumulated response to Selection (R), measured as interline difference and the cumulated Selection differential (S), of the two lines are shown in Fig. 7.

A mean response to selection of 0.43 was obtained by exerting a mean genetic pressure of 2.24 per generation. Selection limit was reached in F_{13}. The mean value of RT calculated in the 13 generations at selection limit was 6.7 ± 1.3.

The heritability realized during the 13 generations of interline separation was 0.21 ± 0.05. It was determined by a least square linear regression of R/S. This value represents the mean h^2 of H and L lines calculated from the interline divergence.

The value of h^2 in each line was influenced by the dominance effect responsible for the asymmetrical response to selection. In relation to the level of the F_0 population (10.1) the RT was 1.4 in H line and 5.3 in L line. The cumulated S was 12.0 and 19.6 respectively. Then h^2 is 0.12 in H line and 0.27 in L line.

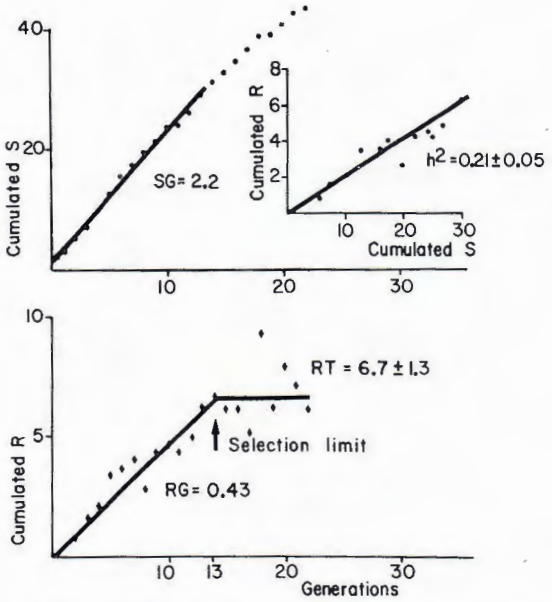

Fig. 7. Genetic analysis of Selection II — cumulated selection differential (S) log 2, in H and L lines — cumulated response to selection (R) log 2, in H and L lines. Calculation of realized heritability (h^2): plot of cumulated R on cumulated S for generations up to selection limit

2. Interline Crosses

The agglutinin responses to SE measured in interline hybrids: F_1, F_2 and in the two backcrosses: BcH and BcL are reported in Table 4 (see also *Biozzi* et al., 1979).

a) Evaluation of the Global Dominance

The dominance of high over low responsiveness measured by the ratio d/a in F_1 hybrids was 0.54. The mean value of dominance measured in F_1, F_2, BcH and BcL was 0.51 ± 0.23. This dominance effect explains why the variance of BcL is larger than that of BcH.

The environmental variance, VE, measured as the mean of the genetically homogeneous population of H, L and F_1 hybrids was 1.31 (Equation 2).

The partition between the genetic and environmental origin of the variance was: in F_2, VG=69% and VE=31%; in BcL, VG=78% and VE=22%. The genetic component of the variability in interline segregant hybrids in therefore larger in Selection II than in Selection I.

The VE values of homozygous generations at selection limit were similar in Selections I and II. The dominance effect of high responsiveness is about 2-fold larger in Selection II than in Selection I. This explains the larger asymmetry in the response to selection observed in Selection II.

Table 4. Mean agglutinin titres and variances in homozygous generations of H and L lines and in interline hybrids of Selection II (14th day post-immunization with the optimal dose of 5×10^8 SE)

Line	Number of mice	Log 2 agglutinin titre	
		Mean $\bar{x}$	Variance V
H line F_{14}–F_{22}	466	11.60	1.01
L line F_{14}–F_{22}	455	4.90	1.47
F_1 (H × L)	88	10.06	1.46
F_2 ($F_1 \times F_1$)	171	9.54	4.22
BcH ($F_1 \times H$)	88	10.27	1.76
BcL ($F_1 \times L$)	146	7.47	5.89
a = 3.35	d = 1.81	d/a = 0.54	

Table 5. Comparison of the different estimates of variance components, heritability and number of loci in Selection II

Environmental variance VE Equation 2	Method of calculation of VA and VD	Partition of genetic variance VG		Heritability h^2 Equation 8	Number of loci n Equation 9
		Additive variance VA	Dominance variance VD		
1.31	Ⓐ From VF_2 and VBcs Equations 5 and 6	0.95	1.94	0.23	5.9
	Ⓑ In VF_2 Equations 3 and 7	2.50	0.38	0.60	2.3
	Ⓒ In VBcs Equations 4 and 7	3.70	0.56	0.66	1.5
	Ⓓ In F_0 Equation 1	0.51	0.61	0.21*	10.9

* Mean value of h^2 realized during the selective breeding (Fig. 6)

b) Evaluation of the Components of the Phenotypic Variance, Heritability and Number of Relevant Loci

The results of the variance analysis of H and L lines and their crosses F_1, F_2, BcH and BcL are reported in Table 5.

The three methods of calculation, A, B and C, give less satisfactory results than those obtained in Selection I (Table 2). Actually, the VA values resulting from calculations B and C are larger than that obtained from calculation A, and give high h^2 values and small numbers of loci. These results greatly contrast with those obtained during the selective breeding. In fact, if the h^2 were 0.60, the selection limit ($RT=6.7$) should have been reached by the 5th generation instead of the 13th as actually observed (Fig. 7).

On the contrary, the h^2 realized during the selective breeding ($h^2=0.21\pm0.05$) is a sound experimental result corresponding to the h^2 evaluated in interline hybrids by calculation A.

The results of calculations A and D are based on independent experimental data, while those of calculations B and C rest upon the assumption implicit in Equation 7. A possible explanation for this discrepancy is given in *Biozzi* et al. (1979).

At present the most probable hypothesis is that the number of independent loci regulating agglutinin responsiveness in Selection II is between six and ten.

The study of linkages with the Ig allotype and with the H-2 locus, and their quantitative contribution to the total interline separation is in progress. It will give experimental evidence in favour of or against the numer of loci hypothesized.

C. Selection for Agglutinin Response to Salmonella Antigen

Two antigenically distinct Salmonellae were alternated at each generation: Salmonella typhimurium (Salm. tm.) and Salmonella oranienburg (Salm. or.). Both Salmonellae contained flagellar (f) and somatic (s) antigens.

Preliminary experiments demonstrated that Salm. tm. and Salm. or. had no cross-reacting antigens in mice and that f and s agglutinin responses were completely independent from each other.

Selective breeding was carried out for the character "peak agglutinin titre" in response to a secondary optimal immunization. The optimal dose was 3.3×10^8 for Salm. tm. and 1×10^9 for Salm. or. Two intraperitoneal injections of formalin-killed bacteria were given 8 days apart. The maximal agglutinin response to both f and s antigens was reached 10 days after the second immunization and measured separately according to the techniques described (*Siqueira* et al., 1976).

From a common F_0 population, two distinct two-way selections were made, one for responsiveness to f antigen (Selection III) and the other for responsiveness to s antigen (Selection IV). Four lines of mice were therefore produced: an H and an L responder line to f antigen, and an H and an L responder line to s antigen. In each selection the agglutinin response to both f and s antigens was measured, one being the "selection antigen" and the other the "associated antigen". Since f and s are completely independent antigens the comparison of the response to the selection antigen and to the associated antigen will give useful information on the specificity of the selective breeding (Chapter IV.C).

The F_0 population common to Selections III and IV was constituted by 75 outbred albino mice obtained from four independent breeding colonies.

1. Selection III for Agglutinin Response to f Antigen of Salmonella

a) Selective Breeding and Genetic Analysis

The F_0 population was immunized with Salm. tm. Then the two Samonellae were alternated at each generation. The individual titres (log 2) of f agglutinins presented a normal distribution in F_0: $\bar{x} = 10.3 \pm 1.21$. The f agglutinin response to Salm. or. was measured in 80 mice of the same origin as the F_0 population. The result was $\bar{x} = 10.9 \pm 1.33$. The equivalence of agglutinin response to Salm. tm. and Salm. or. was confirmed in the successive generations. The data obtained during the selective breeding with both Salmonellae may therefore be cumulated.

A small mean superiority of female responses of about 0.6 log 2 was noticed. The calculations were made from individual data of male and female mice from each generation, both sexes being nearly equally represented.

The mean number of mice per generation was 64 ± 13 in H line and 60 ± 14 in L line. In each line a mean of eight reproductive pairs was selected at each generation.

The results of interline separation produced by 19 consecutive generations of selective breeding are represented in Fig. 8. The phenotypic variance is similar

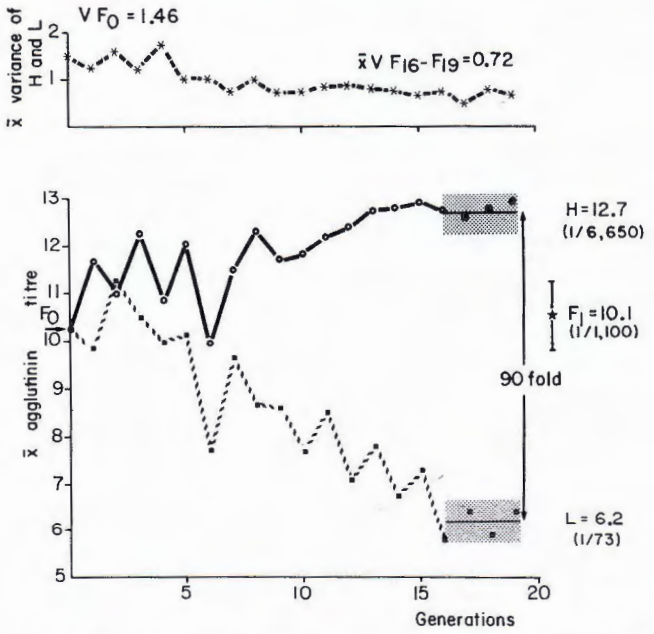

Fig. 8. Selection III. $\bar{x}$ anti f agglutinin titres (log 2) 10 days post 2 i.p. injections (8 days apart) of 3.3×10^8 Salm. tm. or 10^9 Salm. or., in successive generations of H and L lines. F_0–F_{16}: Divergence of H and L lines. F_{16}–F_{19}: Total range of interline separation in homozygous generations $\pm$ standard deviation. Upper part: $\bar{x}$ of H and L variances in corresponding generations

in corresponding generations of each line, therefore their mean value is represented in the upper part of Fig. 8.

H and L responder lines diverged progressively from each other during 16 generations, then in spite of the continuation of the selective breeding up to F_{19} the interline separation remained roughly constant. Selection limit was thus reached in F_{16} (Fig. 9) (*Siqueira* et al., 1976).

The four generations at selection limit, F_{16}–F_{19}, were considered homozygous at the level of all the relevant loci. Their mean response was 12.7 in H line and 6.2 in L line; this makes an interline difference of 90-fold in terms of agglutinin titres. F_1 interline hybrids were produced from F_{19} H and L mice. The f agglutinin response of 86 F_1 was 10.1 ± 0.81. The mean responses of the F_0 population and F_1 hybrids were identical, so in both populations the frequency distribution of the relevant alleles is supposed to be similar and close to the intermediate value of 0.5. The mean agglutinin titre of F_1 was closer to that of H than L homozygous generations, which indicates an incomplete dominance of high over low responsiveness.

The global dominance effect in F_1 hybrids is 20% of the additive effect: $d/a = 0.2$.

The response to selection was asymmetrical. In relation to the level of the F_0 population, agglutinin responsiveness was increased by 5-fold in H line and decreased by 18-fold in L line. Because of the reasons previously discussed, this asymmetrical effect is probably due to the incomplete dominance of the high response.

The mean phenotypic variance produced by environmental factors (VE) measured as the mean of the homozygous generations of H and L lines was 0.72. Then 51% of the variance of the F_0 population is due to genetic factors ($VGF_0 = 0.74$), and 49% to environmental effects.

The results of the selective breeding measured by the interline difference in order to reduce the effect of environmental factors are represented in Fig. 9.

The cumulated values of R or S were plotted in ordinates against the corresponding generations. The mean value of S per generation measured during the interline separation was 2.06. This selective pressure produced a progressive interline divergence at a mean rate of 0.4 per generation until F_{16} when the selection limit was reached. Then the interline difference remained constant in the following generations: $RT = 6.5 \pm 0.5$. The steady rise of cumulated S in the F_{16}–F_{19} generations was therefore entirely due to environmental effects.

The heritability of the character (h^2) measured by a least square linear regression of R/S was 0.20 ± 0.04. This is the mean value of h^2 in both lines calculated from the interline divergence.

Due to the incomplete dominance of high over low response that produced the asymmetrical effect, the h^2 was higher in L than in H line. The h^2 values calculated in each line in relation to the $\bar{x}F_0$ were 0.16 in H line and 0.26 in L line.

An attempt to calculate the number of independent loci affected by the selective breeding, made according to Equation 1, gives $n = 18$. Another estimation of n can be made considering that VGF_0 (0.74) is a maximal value of VA. This is possible since the contribution of VD to VF_0 is negligible due

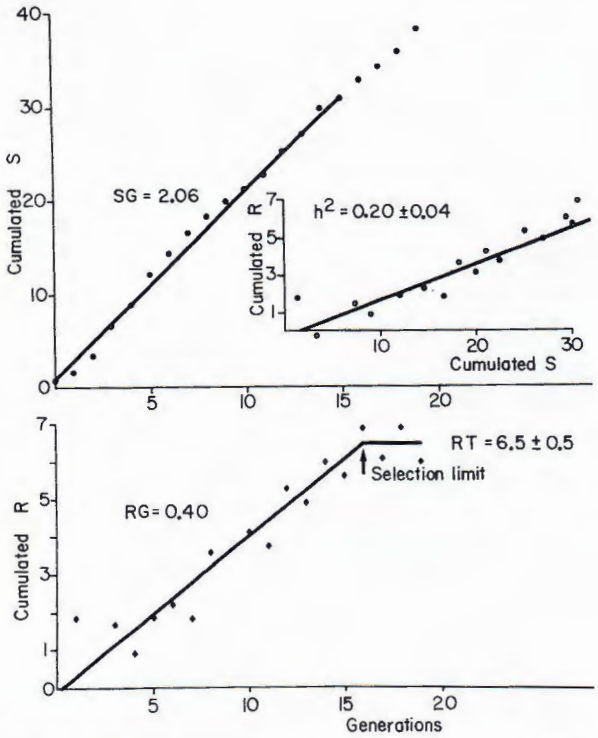

Fig. 9. Genetic analysis of Selection III — cumulated selection differential (S) log 2 in H and L lines — cumulated response to selection (R) log 2 in H and L lines. Calculation of realized heritability (h^2): plot of cumulated R on cumulated S for generations up to selection limit

to the small dominance effect. The consequent estimate of n would be 7 (Equation 9). A study of variance analysis in interline crosses analogous to that made in Selections I and II is in progress.

The data at present available indicate that the number of independent loci regulating the agglutinin responsiveness to f antigen of Salmonella is between 7 and 18.

b) Kinetics of 19S and 7S Agglutinin Production During Primary and Secondary Responses

The phenotypic character chosen for the selective breeding was the maximal secondary agglutinin response to f antigen of Salmonella. The results presented in Fig. 10 demonstrate that the group of genes separated in each line at selection limit also regulates the primary response and operates on the synthesis of both 19S and 7S antibodies.

The kinetics of primary and secondary responses to f antigen of Salm. tm. in H and L lines of Selection III (F_{16}) is represented in Fig. 10. The f agglutinin titre was established either in untreated serum or in ME treated

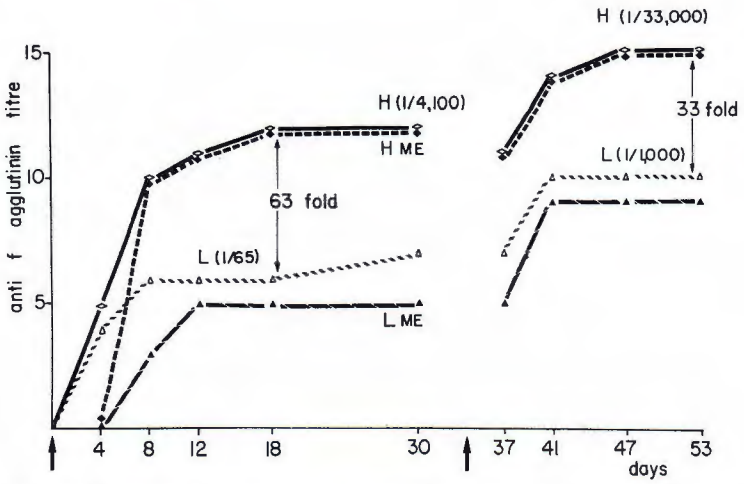

Fig. 10. Kinetics of primary and secondary responses to f antigen of Salm. in H and L lines of Selection III. Total and ME resistant agglutinin titres in groups of six mice from each line immunized i.p. with 3.3×10^8 Salm. tm. on days 0 and 32

serum in order to evaluate the contribution of ME sensitive 19S agglutinins to the total response.

The genetic status of H and L lines operates on both primary and secondary responses. The interline difference at the peak of the primary response was similar to that found for the secondary response of the selective breeding (Fig. 8).

The agglutinin titre in L line persisted at a plateau level until the 30th day, whereas in Selections I and II a rapid decline was observed (Fig. 5).

The primary and secondary responses of H line consisted essentially of ME resistant 7S antibody except during the first 4 days post primary immunization, whereas the contribution of 19S agglutinins to the total response of L line was evident after both primary and secondary immunizations.

2. Selection IV for Agglutinin Response to s Antigen of Salmonella

a) Selective Breeding and Genetic Analysis

The F_0 population was immunized with Salm. tm., then the 2 Salmonellae were alternated at each generation.

The individual titres of s agglutinins presented a normal distribution in F_0: $\bar{x} = 6.4 \pm 1.43$.

The s agglutinin response to Salm. or. measured in a population of 80 mice of the same origin as the F_0 population was $\bar{x} = 6.2 \pm 1.81$, which shows that the two Salmonellae give equivalent responses.

A small mean superiority in female responses of 0.4 was noticed but disregarded in the calculation of means and variances that were established in the total population.

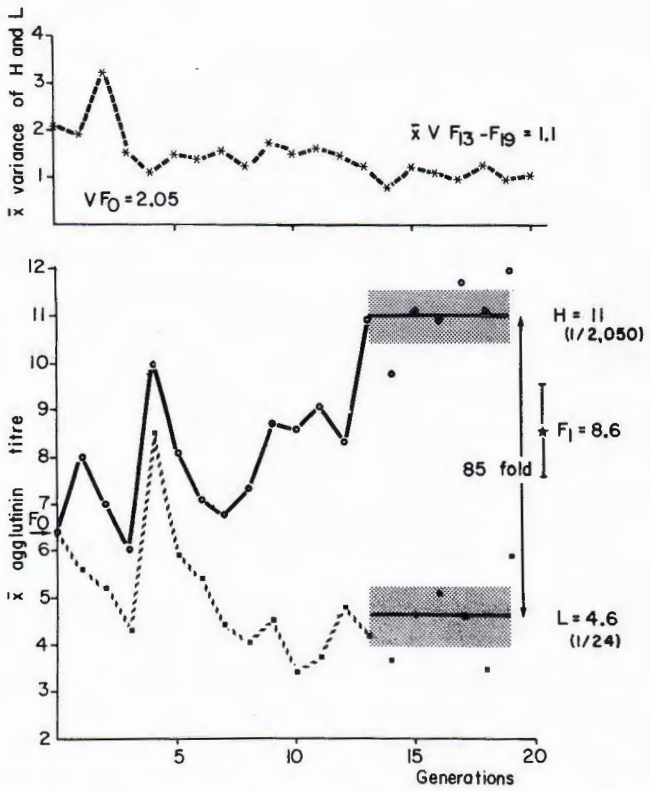

Fig. 11. Selection IV. $\bar{x}$ anti s agglutinin titres (log 2) 10 days post two i.p. injections (8 days apart) of 3.3×10^8 Salm. tm. or 10^9 Salm. or. in successive generations of H and L lines. F_0–F_{13}: Divergence of H and L lines. F_{13}–F_{18}: Total range of interline separation in homozygous generations ± standard deviation. Upper part: $\bar{x}$ of H and L variances in corresponding generations

The mean number of mice per generation was 55 ± 10 in H line and 61 ± 13 in L line. In each line eight to ten reproductive pairs were selected at each generation.

Figure 11 shows the divergence between the mean response of H and L lines produced by 19 consecutive generations of selective breeding.

The selective breeding produced a progressive interline separation accompanied by a concomitant reduction of phenotypic variance during 13 generations. Afterwards the interline separation and the value of the variances remained constant in spite of the continuation of the selection until the 19th generation. Selection limit was therefore reached in F_{13} (Fig. 12) (*Siqueira* et al., 1976).

The mean response of the seven homozygous generations at selection limit was 11.0 in H line and 4.6 in L line. The interline difference was 85-fold in terms of agglutinin titre.

The mean agglutinin response of 73 interline F_1 hybrids produced by mating F_{19} mice was 8.6 ± 0.97.

In F_1 hybrids the frequency distribution of the alleles is supposed to be 0.5, therefore the lowest mean response of the F_0 population is due to a higher frequency of bad effect alleles. The global dominance effect of high responsiveness is 25% of the additive effect: $d/a = 0.25$.

In Selection IV there was also an asymmetrical response but in the opposite direction to that observed in Selection III. In relation to the level of the F_0 population the responsiveness of H line was increased 24-fold while that of L line was decreased only 3.5-fold. In this Selection the asymmetrical response is therefore rather due to the genetic constitution of the F_0 population than to the directional dominance.

The mean environmental variance VE measured in the homozygous generations of H and L lines was 1.08. Then 47% of the phenotypic variance of the F_0 population is due to genetic factors ($VGF_0 = 0.96$) and 53% to environmental effects.

The response to selection R, and the selection differential S, calculated by the interline difference and cumulated from the F_0 population to F_{19} are represented in Fig. 12.

The two lines diverged at a mean rate of 0.48 per generation until F_{13} when the selection limit was reached. This response to selection was produced by a mean selection differential of 2.4 per generation.

The total response to selection RT measured as the mean of the seven generations at selection limit was 6.4 ± 0.5.

The heritability (h^2) calculated by R/S linear regression is 0.21 ± 0.06. Due to the asymmetrical effect the h^2 is higher in H line in which the response to selection is larger than in L line. The h^2 calculated in each line is 0.28 in H line and 0.15 in L line.

The number of independent loci, n, calculated according to Equation 1 is 12.

The minimal estimate of n obtained by postulating that the genetic variance of the F_0 population is entirely produced by additive gene effect is $n = 5$ (Equation 9).

Until the completion of the variance analysis of interline hybrids at present under way, we may conclude that the agglutinin response to s antigen of Salmonellae is regulated by a group of 5–12 independent loci.

b) Kinetics of 19 S and 7 S Agglutinin Production During Primary and Secondary Responses

The total and ME resistant agglutinin response was studied after primary and secondary immunization in H and L lines at selection limit (F_{15}). The results are presented in Fig. 13.

The difference in agglutinin titres between H and L lines was evident in both primary and secondary responses. It should be noted however that the interline difference in secondary response (8-fold) was markedly smaller than that realized at selection limit (85-fold, Fig. 11). This discrepancy may be due to the different immunization schedule used. In the experiments reported in Fig. 13 the booster injection was given 34 days post priming when a high

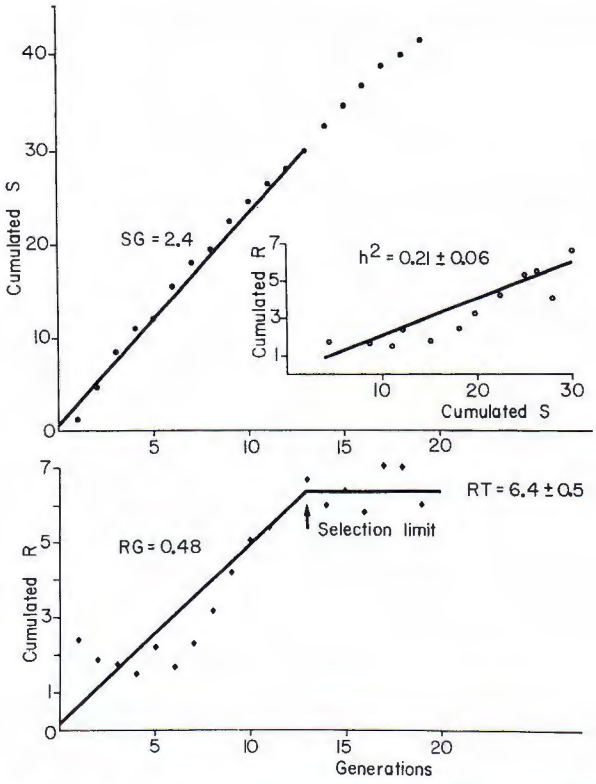

Fig. 12. Genetic analysis of Selection IV — cumulated selection differential (S) log 2, in H and L lines — cumulated response to selection (R) log 2, in H and L lines. Calculation of realized heritability (h^2): plot of cumulated R on cumulated S for generations up to selection limit

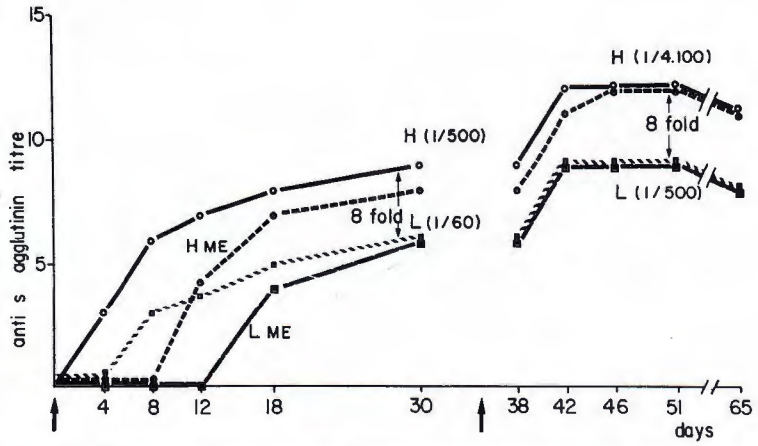

Fig. 13. Kinetics of primary and secondary responses to s antigen of Salm. in H and L lines of Selection IV. Total and ME resistant agglutinin titres in groups of six mice from each line immunized i.p. with 3.3×10^8 Salm. tm. on days 0 and 32

agglutinin level was present in the serum of both lines. In the selective breeding the two injections were only separated by an 8 days interval. At the time of the booster L mice were still unresponsive and H mice had only a low level of serum agglutinin. The regulatory effect of serum antibody on the magnitude of the secondary response may be the cause for the observed discrepancy.

During the initial phase of the primary response only 19S agglutinins were produced in both lines. The contribution of 19S antibody to the total response decreased in the advanced phase of the primary response. After secondary immunization only 7S agglutinins were synthesized in both lines.

These findings underline the importance of the environmental factors, namely the immunization procedure, on the phenotypic effect of the genes regulating immunoresponsiveness.

D. Selection V for Response to Bovine Serum Albumin (BSA) and Rabbit Gamma Globulin (RGG)

1. Selective Breeding and Genetic Analysis

These two non-cross-reacting antigens were alternated at each generation. The first ten generations of selective breeding were immunized with five doses of 2 mg alum precipitated proteins. The first injection was given intravenously, the others subcutaneously, at 5 days interval. The serum antibody titres were measured 8 days after the last injection. An increasing number of non-responder mice were found in L line during the selective breeding, therefore from F_{11} onwards, the two antigens (BSA and RGG) were administered in heat aggregated form in order to increase their immunogenicity. The F_{11} and the successive generations were immunized with two intraperitoneal injections of 1 mg heat aggregated antigens 8 days apart. The antibody response was measured 8 days after the second injection by passive hemagglutination of mouse erythrocytes coupled with BSA or RGG.

The F_0 population consisted of 73 outbred albino mice obtained from four independent colonies. The mean number of mice per generation was 52 ± 16 in H line and 53 ± 11 in L line. Six to nine breeding pairs were culled at each generation.

The mean sex effect, that is a higher responsiveness in females, measured in all the generations, was very small: 0.2 log 2. The means and variances were calculated from individual data of both sexes.

The mean response of the F_0 population immunized with BSA was 6.1 ± 3.9.

The results of the selective breeding are shown in Fig. 14 (*Passos* et al., 1977). The two lines diverged very quickly. In F_6–F_{10} responsiveness in H line rose to a plateau while that in L line remained at a very low level. In these generations many L responders were negative, preventing the correct parental culling. From F_{11} when the two antigens were administered in aggregated form the response was in fact increased to about the same extent in both lines. So, in L line the correct selection of parents was possible again. Nevertheless the interline difference was not larger in F_{11}–F_{16} than in F_6–F_{10}. This means that selection limit was already reached in F_6 (Fig. 15). The increased

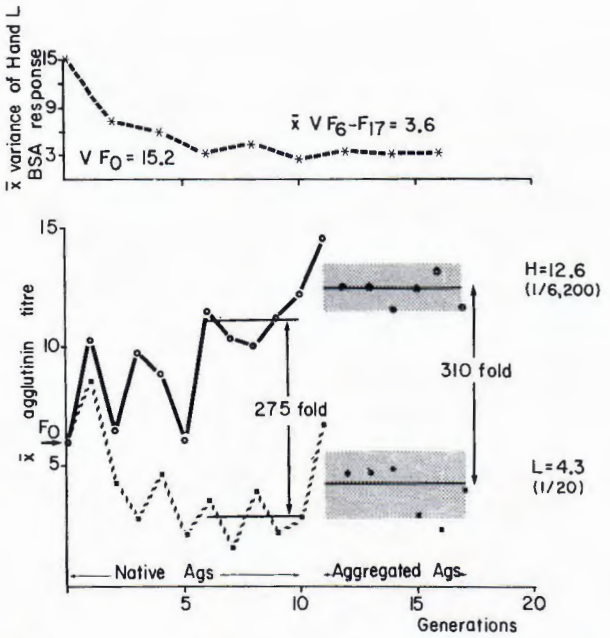

Fig. 14. Selection V. $\bar{x}$ of passive hemagglutination titres (log 2) in successive generations of H and L lines (two immunizations procedures were used, details of which are mentioned in the text). F_0–F_6: Divergence of H and L lines (native antigens). F_6–F_{10}: Total range of interline separation in homozygous generations immunized with native antigens. F_{11}–F_{17}: Total range of interline separation in homozygous generations immunized with aggregated antigens ± standard deviation. Upper part: $\bar{x}$ of variances in corresponding generations of H and L lines (immunized with BSA)

immunogenicity of heat aggregated antigens produced an environmental effect acting in both lines alike without modification of the interline difference.

In F_0–F_{10} generations (immunized with the same procedure) the selective breeding produced an asymmetrical effect. In relation to the level of the F_0 population the agglutinin response of H line was increased 32-fold while it was only decreased 9-fold in L line.

The variances of individual agglutinin titres of the generations immunized with BSA are represented in the upper part of Fig. 14. Since the variances of H and L lines are similar at each generation, the mean value is given.

The variance values decreased rapidly during the interline separation and remained constant in the homozygous generations at selection limit (F_6–F_{17}) when they are only due to environmental effects ($VE = 3.6$). The variance of the F_0 population immunized with BSA was 15.2, therefore 76% of VF_0 was caused by genetic factors ($VGF_0 = 11.6$) and 24% by environmental effects.

The effect of the selective breeding in terms of cumulated R and S calculated from the interline divergence is shown in Fig. 15. The mean agglutinin titres of the generations immunized with BSA and those immunized with RGG at selection limit were similar (Fig. 14). The data obtained in successive generations

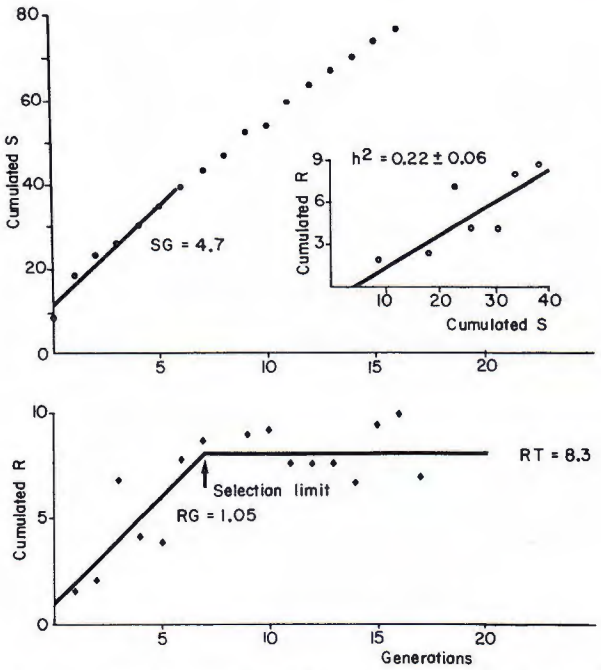

Fig. 15. Genetic analysis of Selection V — cumulated selection differential (S) log 2, in H and L lines — cumulated response to selection (R) log 2, in H and L lines. Calculation of realized heritability (h^2): plot of cumulated R on cumulated S for generations up to selection limit

can thus be directly cumulated to calculate R. Since the difference in the variances of the responses to BSA and RGG was small, the S values of successive generations were also cumulated and S represents the mean value of the genetic pressure relative to the two antigens.

The response to selection was stronger in Selection V than in the preceding four Selections. It was 1.05 per generation, so the selection limit of 8.3 was reached in F_7. This rapid response to selective breeding is due to the large phenotypic variance of the F_0 population, mainly produced by genetic factors (76%). A high selective pressure: SG = 4.7 was exerted. Consequently the heritability ($h^2 = 0.22$) is similar to that observed in the other Selections. In spite of the asymmetrical effect mentioned above (Fig. 14) the heritability is very similar in both lines: h^2 is 0.20 in H line and 0.23 in L line. Unfortunately F_1 hybrids were not tested in the same immunization condition as for the F_0–F_{10} generations, so the effect of the directional dominance on h^2 cannot be established. The mean response in F_1 immunized with heat aggregated BSA is 6.7 ± 3.3. Compared with the response of homozygous parental lines immunized in the same way, this value indicates a global dominance effect of low responsiveness ($d/a = -0.43$).

The strong response to selection produced in few generations of selective breeding and the large values of VF_0 suggest that in Selection V the immune

responses are controlled by a smaller number of loci than in the preceding Selections. In fact, according to Equation 1, only two to three independent loci should intervene. The study of antibody responses in interline crosses is in progress. At present only data obtained in a group of F_1 and F_2 interline hybrids is available. The calculation of n, according to Equations 3, 7 and 9, gives the estimate of three to four loci.

The results of these three estimations obtained from independent data are concordant. They indicate that the quantitative antibody response to the protein antigens used in Selection V is regulated by a group of two to four independent loci.

2. Kinetics of Primary and Secondary Antibody Responses to BSA and RGG

The kinetics of the antibody response to heat aggregated BSA and RGG was studied during the primary response and after a secondary challenge given 35 days later.

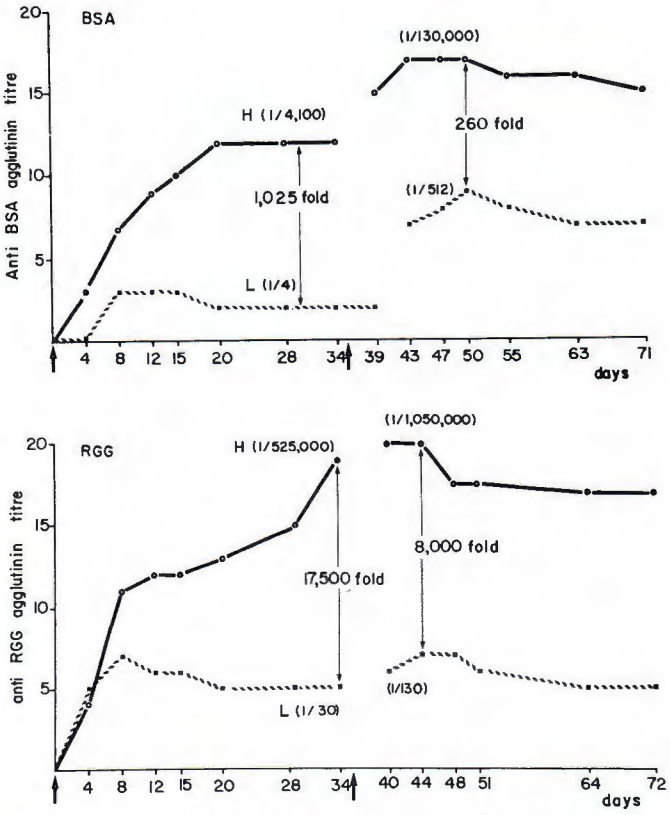

Fig. 16. Kinetics of primary and secondary responses to BSA and RGG in H and L lines of Selection V. Total agglutinin titres in groups of 15 mice from each line, immunized i.p. with 1 mg heat aggregated BSA or RGG on days 0 and 35

The general pattern of primary responses to BSA and RGG was very similar (Fig. 16). In L mice the agglutinin titre reached the peak 8 days post immunization. In H line, on the contrary, there was a progressive increase in antibody titre up to the 20th–34th day post immunization. The interline difference became therefore extremely large at the end of the primary response.

The increase of antibody level after the secondary challenge with RGG was very small compared with the pre-challenge titre, either in H or L line. On the contrary a sharp secondary response to BSA was observed in both lines.

These findings demonstrate that both primary and secondary responses were submitted to the same genetic regulation.

The interline separation obtained in the secondary responses by the immunization schedule used in the experiments reported in Fig. 16 was larger than that produced by the immunization schedule used during the selective breeding (Fig. 14). This stresses the importance of the immunization procedure on the phenotypic expression of the genes.

E. Comparison of the Results of the Five Selections

The five Selections described above were successful. Selection limit was reached after progressive divergence and homozygous H and L responder lines were obtained.

The main genetic parameters of each Selection are summarized in Table 6. These results can be compared since the scale of measurement is identical. The selection procedure was also similar, namely concerning population sizes and selection criteria.

Table 6. Comparison of the genetic analysis of the five Selections

	Selections				
	I	II	III	IV	V
Selection antigens	SE		Salm. tm. & or.		BSA
	PE	SE	f ant.	s ant.	RGG
Foundation Population VP	2.7	2.43	1.47	2.04	15.3
Foundation Population VG/VP %	63	57	52	56	76
Response to selection RT	7.8	6.8	5.8	6.3	8.1
Response to selection RG	0.48	0.42	0.39	0.37	1.05
h_2 [realized heritability]	0.21	0.20	0.18	0.18	0.22
Number of loci Equation 1	15	12	16	12	3
Number of loci Range of other estimates	9–15	2–12	7–18	5–12	2–4
Global dominance: d/a	0.27	0.54	0.20	0.25	−0.43

The differences between the five experiments were restricted to two important factors: the nature of the selection antigen and the procedure of immunization.

The characteristics of the F_0 populations are roughly similar in Selections I, II, III and IV whereas, in Selection V, the phenotypic variance is larger. This difference is essentially due to the genetic component of VF_0. This suggests the intervention of a less complex genetic control, since VF_0, like VF_2, is inversely proportional to the number of loci.

The response to selection is expressed by both RT and RG. RT has the same order of magnitude in the five Selections. This might result from a physiological homeostatic mechanism limiting gene expression. RG is similar in Selections I, II, III and IV in which the maximal interline separation is reached after 13–16 generations of selective breeding. It is clear that the high value of RG in Selection V is due to the large genetic variance of the F_0 population, therefore selection limit is reached in the seventh generation.

The most important parameter to be compared is the realized heritability (h^2) that is close to 0.20 in the five Selections. It must be stressed that the h^2 value depends on the nature of the selected character, especially in relation to fitness. As a rule, higher h^2 values are found for characters of lower adaptive importance. The constant and moderate value of h^2 in our results is understandable, since the quantitative regulation of immune responses is obviously an important component of fitness.

The number of loci responsible for interline difference is indicated first as it results from calculations made with Equation 1. The range of estimates obtained by other methods (presented in detail in the preceding paragraphs) is also reported since the calculation of n is only approximate.

In Selections I, II, III and IV, it may be considered that about ten loci are operating. As anticipated, in Selection V, the number of loci is significantly smaller.

Finally, the d/a ratio, obtained by comparing the responses of F_1 with those of the homozygous parental lines, is also mentioned. The values are positive in Selections I, II, III and IV, which indicates a global dominance effect of high responsiveness. In Selection V on the contrary, the global dominance effect is in the direction of low responsiveness.

IV. Non-Specific Effect of Selective Breeding

The effect of the five selective breedings is not restricted to the antigens used in each Selection (selection antigens). It also operates on antibody responsiveness to many natural immunogens unrelated with the selection antigens. Nevertheless this non-specific effect was not general nor equivalent for all the antigens investigated. In fact the extent of the interline difference in responsiveness to various antigens varied. Sometimes it was as large as that obtained for the selection antigens but it could be smaller for other unrelated antigens, and finally there were some antigens that induced a similar antibody response in H and L lines.

The amplitude of the non-specific effect depends on the nature of the antigens used for the selective breeding and probably also on the immunization procedure.

A. Selection I

In this Selection, the non-specific effect was almost general. The H and L mice were respectively high and low responders to practically all the antigens tested so far (*Biozzi* et al., 1974, 1975). Nevertheless two exceptions have been reported by *Howard* et al. (1974) concerning dextran and levan which induce a similar response in both lines. The two polysaccharides are thymus independent antigens inducing only 19S antibody response. These exceptions are not due to these peculiarities since the Pneumococcus polysaccharide SIII, which has the same characteristics, induces quite different responses in H and L lines (*Howard* et al., 1972). The lack of interline difference in the responses to dextran and levan has been ascribed to the similar rate of their metabolism in the macrophages of H and L responders (*Howard* et al., 1974; *Wiener* and *Bandieri,* 1974).

Apart from dextran and levan, the antibody response to all the other antigens tested was always stronger in H than in L lines whatever the method of immunization used and the adjuvant added. This was demonstrated for the following multideterminant immunogens:

Heterologous erythrocytes: Rat, Horse and Man;

Proteins: hemocyanin (*Unanue* et al., 1974), Bovine serum albumin (*Heumann* and *Stiffel,* 1978), Hen egg albumin (*Prouvost-Danon* et al., 1971);

Haptens: DNP (*Del Guercio* and *Zola,* 1972) and TNP (*Doria* et al., 1978) coupled with various carriers and picryl chloride administered by skin painting (*Mouton* et al., 1974);

Bacterial polysaccharides: Pneumococcus SIII (*Howard* et al., 1972), polysaccharides A and C Streptococcus (Eichmann, personal communication);

Histocompatibility and tumor antigens (*Liacopoulos-Briot* et al., 1972);

Viruses: T_4 bacteriophage (*Howard* et al., 1974) and influenza virus (*Floc'h* and *Werner,* 1978);

Bacteria: flagellar and somatic antigens of Salmonellae (*Sant'Anna* et al., 1979), Streptococci A and C (Eichmann, personal communication), Brucellae (*Cannat* et al., 1978), Yersinia pestis (*Dodin* et al., 1972);

Parasites: Plasmodium berghei (*Biozzi* et al., 1978), Tripanosoma cruzi (*Kierszenbaum* and *Howard,* 1976), Leishmania tropica (Howard, personal communication), Schistosoma donovani (*Blum* and *Cioli,* 1978), Trichinella spiralis (*Perrudet-Badoux* et al., 1975, 1978).

Detailed studies of responses to several unrelated antigens have been made:

1. Salmonella Typhimurium Antigens

The interline difference in agglutinin responses to flagellar (f) and somatic (s) antigens of Salmonella typhimurium (Salm. tm.) is shown in Fig. 17 (*Sant'Anna* et al., 1979).

The kinetics of primary agglutinin response to f and s antigens markedly differ in H and L lines. Thirteen days post-immunization, the interline difference in f and s agglutinin response is 250- and 25-fold respectively. For comparison with the separation obtained for the selection antigens, it should be recalled

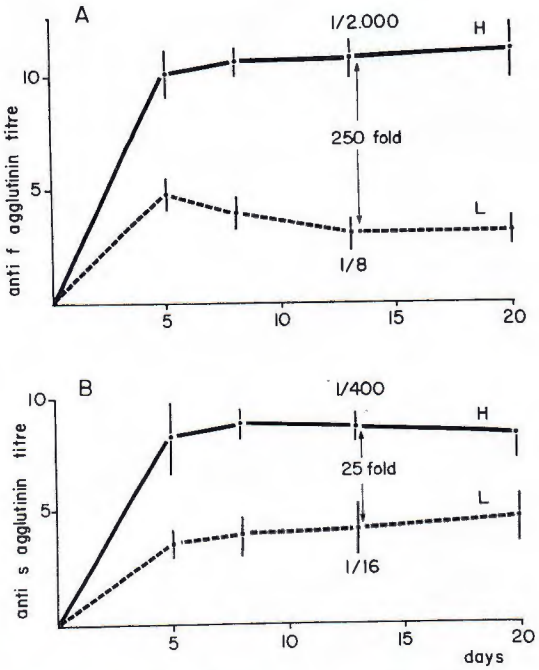

Fig. 17. Kinetics of primary response to f (A) and s (B) antigens of Salm. tm. in H and L lines of Selection I. Agglutinin titres in groups of ten mice from each line immunized i.p. with 3.3×10^8 Salm. tm.

that the interline difference in primary response to SE is 220-fold in Selection I (Fig. 1); 63-fold for the f antigen of Salm. in Selection III (Fig. 10); and 8 fold for the s antigen of Salm. in Selection IV (Fig. 13). The non-specific effect of Selection I for the responsiveness to unrelated Salmonella antigens is thus equal or larger than that concerning the selection antigens used in the three Selections.

2. Bovine Serum Albumin

Heumann and *Stiffel* (1978) demonstrated that the physical state of the antigen and the immunization procedure have an important effect on the level and kinetics of antibody response of H and L lines. An example is given in Fig. 18. Bovine serum albumin (BSA) was injected either intravenously in the form of soluble plurimolecular aggregates (CA-BSA) or intraperitoneally in the form of alum precipitates (Alum-BSA).

The intravenously injected CA-BSA was rapidly cleared from the circulation by liver and spleen macrophages. The antibody response was therefore induced in the spleen by a single antigen pulse. On the contrary, after intraperitoneal immunization, the alum precipitated BSA induced a continuous and protracted antigenic stimulation.

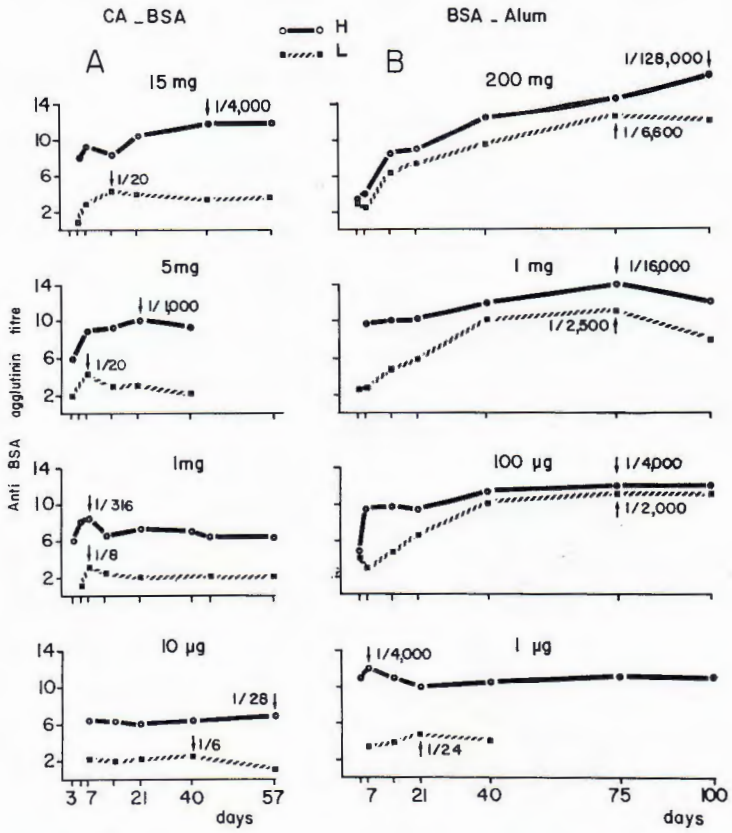

Fig. 18. Kinetics of primary response to increasing doses of BSA in H and L lines of Selection I. A. Heat aggregated BSA: CA-BSA injected i.v. B. Alum precipitated BSA injected i.p. in groups of 10–20 mice from each line

The antibody response to all doses of CA-BSA was markedly stronger in H than in L mice. For the largest antigen dose (15 mg) the interline difference was 200-fold. This non-specific effect corresponds to the interline difference observed for the optimal dose of selection antigen (SE) that is 220-fold (Fig. 1). Both SE and CA-BSA antigens injected intravenously produced a single pulse of immunogenic stimulation in the spleen.

The difference in antibody response between H and L mice immunized intraperitoneally with alum precipitated BSA was reduced, in particular in the advanced phase of the response to the largest antigen doses.

The different results obtained with the two immunization procedures can be explained by the genetic modifications of the antigen catabolism in macrophages described in Chapter V.B. The persistence of the antigen in macrophages is a limiting factor of antibody response after the single pulse of antigen produced by intravenous immunization (*Biozzi* et al., 1972a). On the contrary, this limiting factor may be partially overwhelmed when the intraperitoneal immunization with the adjuvant precipitated antigen provides a continuous antigenic supply.

Table 7. Induction of immunological memory to BSA: Secondary responses to CA-BSA in groups of H and L mice primed with increasing doses of CA-BSA

Priming dose µg	Secondary* challenge µg	Peak agglutinin titre H line	L line
—	10	360	6
0.01	10	128	—
0.1	10	16000	—
1	10	4000	32
10	10	8000	8
100	10	4000	1024

* Two months after priming

This is a typical example of the modification of the phenotypic expression of the genotype by an environmental factor.

The induction of immunological memory to BSA was also affected by the genes regulating immunoresponsiveness in H and L lines as shown in Table 7. In H line the minimal priming dose was 0.1 µg whereas a 1000-fold higher dose (100 µg) was required to induce the immunological memory in L line.

The modification of the threshold immunizing dose of selection antigen described in chapter III.A (Fig. 3) was also observed for unrelated antigens such as BSA and hen egg albumin. The minimal dose of CA-BSA giving a detectable response was 10^{-3} µg in H line and 100 µg in L line, this makes a 10000-fold difference. This effect is larger than that observed for the selection antigen which is about 100-fold (Fig. 3).

3. Hen Egg Albumin

A detailed study of antibody response to hen egg albumin (EA) was made in H and L lines and their hybrids by *Prouvost-Danon* et al. (1977). A wide range of alum precipitated EA doses was used with the emphasis on small doses since the responsiveness to limiting doses of EA is controlled, in inbred mice, by a specific Ir gene (*Vaz* et al., 1971).

The results obtained in H, L and F_1 hybrids, reported in Fig. 19, show some characteristics already observed in the study of responsiveness to other antigens: SE (Fig. 3) and CA-BSA. The sensitivity to antigen stimulation is higher in H than in L line: the threshold immunizing EA dose is about 100-fold lower in H than in L mice.

The interline difference, which was very large for low EA doses, decreased as the antigen dose was increased since the immunization was given by intraperitoneal route with alum precipitated antigen.

An important result was obtained concerning the relationship between the antigen dose and the dominance effect in the F_1 hybrids. High response character, which is dominant for the dose of 1 µg, became progressively recessive as the antigen dose decreased, and was completely recessive for the dose of 0.05 µg.

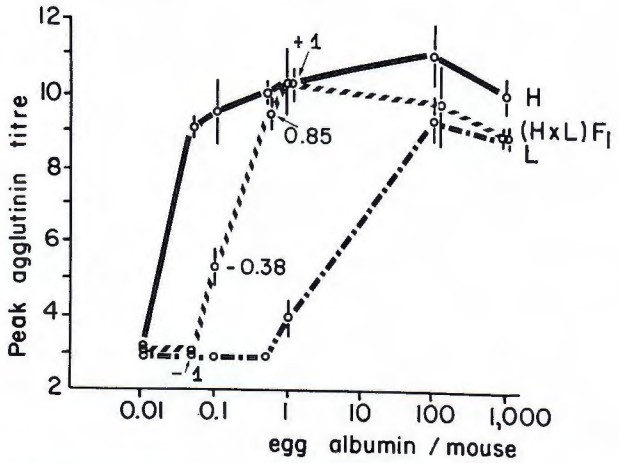

Fig. 19. Dose-response to egg albumin (EA) in H and L lines of Selection I and their F_1 hybrids. Peak values of passive hemagglutination titres in groups of 8–15 mice immunized i.p. with increasing doses of alum precipitated EA. Figures close to F_1 values are d/a values for corresponding doses

Table 8. Observed versus theoretically expected percentages of responder mice in interline hybrids immunized with 0.05 µg EA, according to a one-locus model hypothesis

	Number of mice	Responder mice (agglutinin titres > 4 log 2)			
		Observed		Expected	
		number of mice	%	number of mice	%
H	13	13	100		
L	10	2	20		
F_1	5	0	0		
F_2	46	6	13	14	30
BcH	18	11	61	9	50
BcL	5	0	0	0.5	10

The inheritance of responsiveness to the threshold EA dose of 0.05 µg, that permits a clear discrimination between responders and non-responders, was studied in F_1, F_2 and both backcrosses.

The distribution of parental phenotypes, responder or non-responder, in interline hybrids, was compared with the theoretical distribution based on a single locus hypothesis and the observed data, reported in Table 8, proved to be close to the expected ones, which demonstrates that a single locus operates. The variance analysis of larger groups of mice is in progress. The preliminary results confirm the monogenic control of responsiveness to this EA dose.

It was particularly interesting to know whether or not this single locus operating at the threshold EA dose was H-2 associated. Consequently the anti-

body responsiveness was studied in groups of F_3 H/H and F_3 L/L mice. As described in chapter III.A.3, these mice are homozygous for the H-2 phenotype of H and L mice respectively on an identical background equivalent to that of F_2 mice. The results show that all F_3 H/H mice were responders, as H mice are, whereas all F_3 L/L mice were non-responders, as L mice are (*Mouton* et al., 1977).

The paramount importance of an H-2 linked gene in the control of antibody response to threshold EA and SE (Chapter III.A) doses in H and L lines is well established. This gene participates also in the regulation of responsiveness to optimal doses of antigen but in this case its quantitative importance is reduced since the control becomes polygenic.

B. Selection II

In this Selection the non-specific effect was less extensively studied than in Selection I; nevertheless, the data summarized in Table 9 clearly indicate a concomitant modification of antibody responsiveness to five immunogens unrelated with the selection antigen (SE).

The results are expressed in terms of interline differences of log 2 agglutinin titres or of agglutinin titres ratios.

The antibody responses to all the unrelated antigens are markedly higher in H than in L line, although the difference is always smaller than that concerning the selection antigen (34%–65%).

C. Selections III and IV

In these two Selections the importance of the non-specific modification of antibody responsiveness produced by the selective breeding was studied throughout the selective process, from the F_0 population to selection limit (*Siqueira* et al., 1977). The Salmonella contains two independent antigens, f and s. In each Selection the agglutinin response was measured for the two antigens. The response to Selection, measured in terms of progressive interline difference for the selection antigen, was accompanied by an equivalent effect on the response to the associated antigen, as shown in Fig. 20.

The interline difference in the agglutinin response to the selection antigen is represented, in abscissae, against the same effect measured for the associated antigen in all the generations from the F_0 population to selection limit, in ordinates. The slope measured by a least square linear regression is 0.97 in Selection III and 0.94 in Selection IV. Thus the modification of responsiveness to the associated antigen is almost equivalent to that realized for the selection antigen. The non-specific effect is observed from the beginning of the selective breeding and increases until selection limit, which indicates that the same genes accumulated progressively in each line during the selective process regulate the antibody responsiveness to the two unrelated antigens f and s. The progressive interline separation for antibody responses to other antigens: SE, DNP-HGG and BGG measured at different stages of the selective breeding was

Table 9. Non-specific effect in Selection II — Maximal agglutinin titres in H and L mice immunized with various unrelated antigens — Comparison of interline difference with that observed for selection antigen

Antigen	Agglutinin response				p	Interline difference			Number of mice	Generation tested
	H line		L line			H–L Log 2	H/L Aggl. titre	% of Selection Ag.		
	Log 2	Aggl. titre	Log 2	Aggl. titre						
Selection Ag. Sheep erythrocytes (SE)	11.6±1.0	3100	4.9±1.2	30	<0.001	6.7	103	100	466 H 455 L	F_{14}–F_{22}
Pigeon erythrocytes (PE)	14.3±2.0	20160	9.9±1.3	955	<0.02	4.4	21	65	5 H 5 L	F_{14}
Hen egg albumin (EA)	8.1±1.0	274	4.9±0.9	30	<0.001	3.2	9	48	6 H 6 L	F_{17}
f antigen of Salm. tm.	10.4±1.6	1350	6.4±0.7	84	<0.001	4.0	16	60	8 H 8 L	F_{23}
s antigen of Salm. tm.	6.7±1.6	104	4.4±0.6	21	<0.01	2.3	5	34	8 H 8 L	F_{23}
Plasmodium berghei	13.5±0.6	11600	10.5±0.7	1450	<0.001	3.0	8	44	9 H 12 L	F_{18}

SE = 14th day after i.v. injection of 5×10^8.
PE = 14th day after i.v. injection of 1×10^8.
EA = 21st day after i.p. injection of 1 µg alum precipitated.
Salm. tm. f = 21st day after i.p. injection of 3.3×10^8 Salm. tm.
Salm. tm. s = 21st day after i.p. injection of 3.3×10^8 Salm. tm.
P. berghei = 7 days after 6 i.p. injections of 3×10^7 parasitized erythrocytes irradiated at 60000 r (immunofluorescent assay).

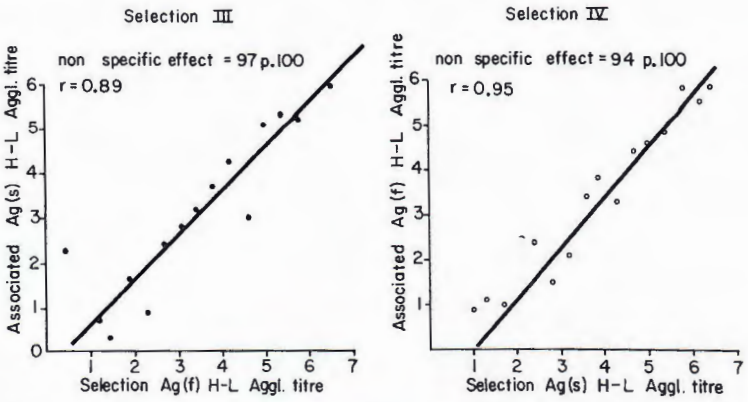

Fig. 20. Correlation between interline differences in responses to selection antigen and associated antigen in Selections III and IV. Difference between means of peak agglutinin titres to both antigens in successive generations of the two Selections (correlation calculated by a least square regression curve)

also demonstrated in Selections III and IV with a very important exception concerning Selection IV. In this Selection an equivalent agglutinin response of H and L lines to SE was demonstrated in F_4, F_6, F_8, F_{10}, F_{12} and F_{14} (*Siqueira* et al., 1977).

The antibody response to ten unrelated antigens tested in H and L lines of Selection III at selection limit is reported in Table 10 and compared with the interline difference concerning the selection antigen f considered as 100%.

The almost equivalent non-specific effect (92%) on the responsiveness to the associated antigen s observed during the interline separation (Fig. 20) was confirmed.

A very large interline difference was also observed in the agglutinin response to Salmonella anatum especially for the s antigen. In previous experiments carried out in H and L mice it was clearly demonstrated that Salm. tm. and Salm. anatum were completely antigenically unrelated in terms of immunogenic and antigenic characteristics of both f and s antigens.

Another strong non-specific effect concerns BGG, since the L line is almost completely unresponsive to this antigen. An intermediate non-specific effect is observed for the other antigens. The interline difference in antibody responses to DNP hapten coupled to BGG or HGG was noticeable, though smaller, than that obtained with the two native carriers.

A very important exception concerns the agglutinin response to pigeon erythrocytes since there was no significant difference between H and L lines.

The non-specific modification of the antibody responses to 11 unrelated antigens in H and L lines of Selection IV is represented in Table 11.

In this selection the non-specific effect was smaller and less extensive than that produced in Selections I, II and III. It was very pronounced for some antigens, like the associated f antigen and the unrelated f and s antigens of Salm. anatum, intermediate for other antigens such as BGG and BSA, small

Table 10. Non-specific effect in Selection III — Maximal agglutinin titres in H and L mice immunized with various unrelated antigens — Comparison of interline difference with that observed for selection antigen

Antigen	Agglutinin response				p	Interline difference			Number of mice	Generation tested
	H line		L line			H–L Log 2	H/L Aggl. titre	% of Selection Ag.		
	Log 2	Aggl. titre	Log 2	Aggl. titre						
Selection Ag. f antigen Salm. tm. and Salm. or.	12.7±0.8	6650	6.2±0.9	73	<0.001	6.5	91	100	236 H 205 L	F_{16}–F_{19}
Associated Ag. s antigen Salm. tm. and Salm. or.	10.8±1.1	1780	4.8±1.3	27	<0.001	6.0	66	92	236 H 205 L	F_{16}–F_{19}
s antigen of Salm. anatum	9.3±1.7	630	2.4±1.0	5	<0.001	6.9	126	106	15 H 15 L	F_{16}
f antigen of Salm. anatum	11.6±1.0	3100	7.1±0.8	140	<0.001	4.5	22	69	15 H 15 L	F_{16}
Bovine gamma globulin (BGG)	8.9±2.3	477	2.9±2.1	7	<0.001	6.0	68	92	16 H 16 L	F_{18}
DNP_{22}–BGG	11.7±1.3	3330	7.6±0.6	193	<0.001	4.1	17	63	14 H 14 L	F_{16}
Bovine serum albumin (BSA)	8.8±2.2	445	4.1±1.0	17	<0.001	4.7	26	72	15 H 15 L	F_{18}
Human gamma globulin (HGG)	9.5±1.9	720	5.6±1.4	48	<0.001	3.9	15	60	15 H 15 L	F_{16}
DNP_{27}–HGG	10.6±1.4	1550	7.8±0.8	220	<0.001	2.8	7	43	15 H 15 L	F_{16}
Sheep erythrocytes (SE)	11.6±1.5	3100	7.9±1.2	238	<0.001	3.7	13	57	16 H 16 L	F_{16}
Pigeon erythrocytes (PE)	9.7±1.7	830	9.1±1.0	548	n.s.	0.6	1.5	9	15 H 15 L	F_{16}

Salm. = 10–12 days after 2 i.p. injections (8 days apart) of 3.3×10^8 Salm. tm. or 10^9 Salm. or. and Salm. anatum.

BGG, HGG = 10 days after the third injection of 1 mg alum precipitated BGG or HGG the first given i.v. the two others i.p. on days 0, 4 and 8.

DNP–HGG, DNP–BGG = 12 days after i.p. injection of 1 mg in CFA.

BSA = 10 days after two i.p. injections (8 days apart) of 1 mg (heat aggregated).

SE = 12 days after i.v. injection of 5×10^8.

PE = 13 days after i.v. injection of 1×10^8.

Table 11. Non-specific effect in Selection IV — Maximal agglutinin titres in H and L mice immunized with various unrelated antigens —

BGG, HGG } = itated BGG or HGG the first given i.v. the two others i.p. on days 0, 4 and 8.

SE = 12 days after i.v. injection of 5×10^8.
PE = 13 days after i.v. injection of 1×10^8.

Table 11. Non-specific effect in Selection IV — Maximal agglutinin titres in H and L mice immunized with various unrelated antigens — Comparison of interline difference with that observed for selection antigen

Antigen	Agglutinin response				p	Interline difference			Number of mice	Generation tested
	H line		L line			H–L Log 2	H/L Aggl. titre	% of Selection Ag.		
	Log 2	Aggl. titre	Log 2	Aggl. titre						
Selection Ag. s antigen Salm. tm. and Salm. or.	11.0±1.0	2048	4.6±1.2	24	<0.001	6.4	85	100	352 H 361 L	F_{13}–F_{19}
Associated Ag. f antigen Salm. tm. and Salm. or.	12.7±1.0	6650	7.0±1.1	128	<0.001	5.7	52	89	352 H 361 L	F_{13}–F_{19}
DNP Salm. tm.	14.7±0.8	26600	9.8±2.9	890	<0.01	4.9	30	76	6 H 6 L	F_{15}
f antigen Salm. anatum	12.4±0.9	5400	7.0±0.7	128	<0.001	5.4	42	84	16 H 16 L	F_{18}
s antigen Salm. anatum	9.7±1.4	830	5.1±1.5	34	<0.001	4.6	24	72	16 H 16 L	F_{18}
Bovine gamma globulin (BGG)	6.7±2.4	104	3.5±1.9	11	<0.02	3.2	9	50	12 H 12 L	F_{16}
Bovine serum albumin (BSA)	9.1±2.7	550	6.2±2.4	73	<0.05	2.9	8	45	15 H 17 L	F_{18}
Pigeon erythrocytes (PE)	10.4±2.0	1350	8.9±1.6	480	<0.05	1.5	3	23	14 H 15 L	F_{21}
DNP_{27}–HGG	10.9±0.5	910	10.2±0.8	1176	=0.01	0.7	1.6	11	14 H 14 L	F_{16}
Human gamma globulin (HGG)	5.2±1.5	37	4.7±1.3	26	n.s.	0.5	1.4	8	14 H 16 L	F_{18}
Sheep erythrocytes (SE)	9.1±1.1	550	10.1±0.7	1100	=0.01	−1	0.5	−15	14 H 14 L	F_{16}
Human erythrocytes (HE)	5.8±2.1	55	6.9±2.0	120	n.s.	1.1	0.46	−17	11 H 11 L	F_{18}

Salm. = 10–12 days after 2 i.p. injections (8 days apart) of 3.3×10^8 Salm. tm. or 1.10^9 Salm. or. and Salm. anatum.
DNP–Salm. = 12 days after 2 i.p. injections (10 days apart) of 5×10^8.
BGG, HGG } = 10 days after the third injection of 1 mg alum precipitated BGG or HGG, the first given i.v., the other two i.p. on days 0, 4 and 8.
DNP–HGG = 12 days after i.p. injection of 1 mg in CFA.
BSA = 10 days after 2 i.p. injections (8 days apart) of 1 mg (heat aggregated).
SE = 12 days after i.v. injection of 5×10^8.
PE = 13 days after i.v. injection of 1×10^8.
HE = 8 days after i.v. injection of 1×10^8.

but significant for PE and DNP hapten coupled to Human gamma globulin (HGG). No significant difference between H and L lines was observed in the antibody responses to Human erythrocytes (HE) and HGG. Moreover the agglutinin response to SE was slightly but significantly stronger in L than in H line.

D. Selection V

The non-specific effect of Selection V was studied by immunizing H and L mice with five immunogens unrelated with the selection antigens, as indicated in Table 12.

The antibody responses of each line to the selection antigens BSA and RGG in the F_1–F_{17} generations were similar, therefore the interline difference in the antibody responses to the unrelated antigens mentioned in Table 12 are compared to the mean of the interline separation for BSA and RGG responses considered as 100%.

In preliminary experiments, it was proved that chicken gamma globulin (CGG) is immunogenically and antigenically completely unrelated with RGG. An important non-specific effect was observed in the response of H and L mice to this antigen. The interline difference was very close to that concerning the selection antigens. A small but significant non-specific effect was also observed for the antibody response to SE and f antigen of Salm. tm., but this effect was only about 13% of that found for the selection antigens. The responsiveness to s antigen of Salm. tm. and to EA was equal in H and L lines.

Results in Table 12 show that in Selection V a strong non-specific effect is only observed for one antigen out of the five so far studied.

The comparison of the results obtained in the five Selections described above indicates that the non-specific effect of the relevant genes of each selective breeding differs in both quantitative and qualitative expressions. It seems to be very broad and efficient in Selections I and III, limited and weak in Selection V, and intermediate in the other Selections (II and IV).

These differences are not yet clearly understood. They most probably depend on the complexity of the selection antigen or the immunization procedure used rather than on the difference in the genetic properties of the F_0 populations. In fact Selections III and IV are founded on the same F_0 population and Selection V on a F_0 population of the same origin as that of Selections III and IV. A plausible hypothesis, deriving from the experimental results is that the extent of the non-specific effect depends on the number of independent loci involved in each selective breeding. When a large number of loci are concerned, as for Selection I ($n=1.0$) and Selection III ($n=7$–18) the non-specific effect is broad, whereas for Selection V in which only three to four loci are concerned, the non-specific effect is reduced.

It may therefore be hypothesized that the number of genes required for the regulation of antibody responsiveness is inversely proportional to the antigenic complexity of the immunogen. This explains why a single specific Ir gene controls the response to synthetic polypeptides of limited molecular complexity (*Benacerraf* and *McDevitt*, 1972).

Table 12. Non-specific effect in Selection V — Maximal agglutinin titres in H and L mice immunized with various unrelated antigens — Comparison of interline difference with that observed for selection antigen

Antigen	Agglutinin response				p	Interline difference			Number of mice	Generation tested
	H line		L line			H–L Log 2	H/L Aggl. titre	% of Selection ag.		
	Log 2	Aggl. titre	Log 2	Aggl. titre						
Selection antigens										
Bovine serum albumin (BSA)	12.8±1.9	7130	4.6±2.4	24	<0.001	8.2	297	100	202 H	F_{11}–F_{17}
						} 8.3	} 317			
Rabbit gamma globulin (RGG)	12.4±2.1	5400	4.0±1.9	16	<0.001	8.4	337		214 L	
Chicken gamma globulin (CGG)	13.8±1.4	14260	6.1±2.1	68	<0.001	7.7	209	93	15 H 14 L	F_{17}
Sheep erythrocytes (SE)	9.4±0.8	675	8.4±1.1	337	<0.02	1.0	2	12	21 H 18 L	F_{15}
f antigen of Salm. tm.	10.8±0.9	1780	9.7±0.8	830	=0.001	1.1	2.1	13	15 H 15 L	F_{15}
Hen egg albumin (EA)	16.4±5.1	86450	15.2±4.0	37630	n.s.	1.2	2.3	14	14 H 14 L	F_{16}
s antigen of Salm. tm.	5.6±1.1	48	5.6±0.9	48	n.s.	0	1	0	15 H 15 L	F_{15}

BSA, RGG, CGG = 8–10 days after two i.p. injections (8 days apart) of 1 mg (heat aggregated).

SE = 12 days after 5×10^8.

Salm. tm. = 10–12 days after 2 i.p. injections (8 days apart) of 3.3×10^8.

EA = 8 days after 5 injections of 2 mg (alum precipitated), the first given i.v., the others i.p., 5 days apart.

V. Cellular Expression and Functions of the Genes Regulating the Quantitative Antibody Response

The findings reported in this chapter were obtained in mice of Selection I, the most extensively studied Selection.

The group of genes accumulated in each line by selective breeding regulates the activity of the immunocompetent cells themselves, namely lmyphocytes and macrophages. The results of the in vitro response reported later, exclude the relevant intervention of systemic mechanisms of regulation outside the lymphoid tissue.

A. Phenotypic Expression at Lymphocyte Level

The genetic modification of lymphocyte potentiality was demonstrated in vivo by adoptive immunization experiments.

Irradiated immuno-suppressed random bred mice or $(H \times L)F_1$ hybrids were restored with an equivalent number of spleen cells or lymph node cells from H or L donors and then immunized with SE. The SE agglutinin response was always much stronger in mice restored by H line cells than by L line cells. Similar results were obtained with lymphocyte populations deprived of macrophages by surface adherence on a glass beads column (*Biozzi* et al., 1974).

These results demonstrate the different potentialities of lymphocytes of H and L line mice. Similar observations have been made in mice of Selection III (M. Siqueira, personal communication).

A criticism that may be made of this type of experiments is that a graft-versus-host reaction occurs concomitantly with adoptive response. However, since the T cell mediated response is similar in both lines (paragraph E) this criticism does not contradict the essential significance of these experiments that is furthermore confirmed by the results of in vitro immunization (paragraph D).

The finding that response to thymus independent antigens such as Pneumococcus polysaccharide SIII is stronger in H than in L mice indicates that B lymphocytes are concerned by the genetic control (*Howard* et al., 1972). The possible regulatory helper or suppressor effect of T lymphocytes remains to be investigated.

B. Phenotypic Expression at Macrophage Level

The principal functions of macrophages are: antigen phagocytosis, antigen processing and participation in the mechanism of cell interactions required for antibody response.

The phagocytic function of macrophages was not affected by the selective breeding. The rate of phagocytosis of carbon particles and of Cr^{51} labelled SE by the liver and spleen macrophages is similar in H and L lines. The same amount of SE was phagocytized by spleen macrophages of both lines

2 hours after the injection of this antigen by intravenous route (*Mouton* et al., 1976). On the contrary, the rate of intracellular catabolism of phagocytized SE was rapid in L macrophages and slow in H macrophages.

The persistance of SE in immunogenic form in spleen macrophages was studied as follows: Primed random bred mice were immunized with homogenized and irradiated spleens removed from either H or L mice at various times after immunization with SE by intravenous route. The persistance of SE antigen in spleen was revealed by the agglutinin response of the recipients. Similar agglutinin titres were obtained when the spleens were removed 2 hours post SE injection. After 4 days the SE antigen was no longer detectable in L line spleens whereas it persisted for 2 weeks in H mice spleens (*Biozzi* et al., 1974). This finding was confirmed by the measure of the rate of Cr^{51} release from macrophages fed in vitro with equivalent numbers of Cr^{51} labelled SE (*Mouton* et al., 1976).

Another proof of the genetic modification of the macrophage function resulting from the selective breeding is given by the following in vivo experiment. The same number of spleen cells from F_1 hybrids was injected in X-ray immunosuppressed H or L recipients that were then immunized with SE. The SE agglutinin response was 1/1280 in H recipients and 1/80 in L recipients. This difference underlines the importance of the radioresistant macrophage functions in the antibody responsiveness of H and L mice (*Biozzi* et al., 1974).

Similar results were obtained by *Wiener* and *Bandieri* (1974) with another antigen: keyhole limpet hemocyanin. They observed that the amount of antigen bound to the membrane of L mice macrophages decreased rapidly while it persisted for a long time on the membrane of H mice macrophages. Furthermore the lysosomal enzyme activity was found to be higher in L than in H mice macrophages.

The difference in the enzyme equipment of H and L mice macrophages has a very marked influence on the survival and multiplication of ingested micro-organisms. The bactericidal activity is much stronger in L than in H mice macrophages. This difference was demonstrated for a variety of micro-organisms such as: T_4 bacteriophage (*Howard* et al., 1974), Salmonella, Lysteria monocytogenes (Fauve, personal communication), Mycobacterium tuberculosis and Mycobacterium leprae murium (Lagrange, personal communication), Brucella suis (*Cannat* et al., 1978) and Leishmania tropica (Howard, personal communication).

All these observations demonstrate that the metabolic activity of macrophages is an important physiologic parameter in the regulation of antibody responsiveness. This is in agreement with a recent report by *Ishizaka* et al. (1978). In H and L lines, this regulation modifies the persistance of the immunogenic form of the antigen in the macrophages. A slow destruction in H line macrophages induces a long lasting stimulation of the lymphocytes and then a high antibody response. The rapid antigen breakdown in the L mice macrophages is an important cause of their low antibody response. The inverse relationship between macrophage activity and antibody responsiveness is very important, to understand the mechanism of the anti-infection immunity described in Chapter VI.

C. Cytodynamics of the Immune Response

The early exponential phase of immune response after intravenous immunization with SE was studied at cellular level by determining the number of plaque forming cells (PFC) and Rosette forming cells (RFC) in the spleens of H and L mice (*Biozzi* et al., 1971, 1972a). The principal conclusions are the following:

— The difference between H and L responders is mainly due to the number of antibody forming cells, rather than to the amount of antibody released by each single cell.

— The rate of multiplication and differentiation of the clones of specific cells induced by antigen stimulation is very different in the two lines: the mean doubling time of RFC is 9 hours in H line and 16 hours in L line.

— The target cells for SE are small lymphocytes. Their number is similar in H and L lines (about 4000). The differentiation rate of this population of small lymphocytes into blast cells and finally into plasmocytes is much more rapid in H than in L mice. At the end of the exponential phase (4th day) there are 650000 RF plasmocytes per spleen in H responder mice and only 15400 in L responder mice. The H/L ratio of RF plasmocytes per spleen is identical to that of serum agglutinin titres on the same day (*Biozzi* et al., 1972b).

Other important immuno-biological characteristics of H and L lines of Selection I are the following:

— The difference in serum antibody levels between H and L responders is due to the genetic modification of the rate of antibody synthesis, whereas the metabolic decay of antibody molecules is similar in both lines (*Oriol* et al., 1972).

— The group of genes accumulated in the two lines by selective breeding, regulates the synthesis of all the classes of immunoglobulins: IgM, IgG (Fig. 5), IgG_1, IgG_2 (*Biozzi* et al., 1970; *Lieberman* et al., 1972), Reagins (*Prouvost-Danon* et al., 1971, 1977) and IgA (*André* et al., 1977).

— The total serum concentration of Ig is lower in L than in H line. This difference increases markedly after immunization and concerns all the Ig classes, as shown in Table 13.

Table 13. Levels of classes and sub-classes of immunoglobulins in H and L mice of Selection I before immunization or 14 days after i.v. injection of 5×10^8 SE

	Serum immunoglobulins (mg/ml)			
	Before immunization		After immunization	
	H line	L line	H line	L line
IgM	0.17	0.14	0.40	0.14
IgG1	0.78	0.31	3.60	0.24
IgG2	1.45	0.61	6.00	0.75
IgGA	0.54	0.34	0.90	0.46
Total	2.94	1.40	10.90	1.59

On the whole, L mice are genetically hypoglobulinemic. This explains their poor general antibody responsiveness to all antigens (*Biozzi* et al., 1970).

D. In Vitro Immune Response

The following experiments were carried out by *Doria* et al. (1978).

The antibody response was measured as the number of direct Plaque Forming Cells (PFC) obtained in spleen cell cultures immunized in vitro with SE according to the Mishell and Dutton method (*Mishell* and *Dutton*, 1967).

In each experiment, cells of H and L lines were simultaneously tested in order to eliminate the effect of the large variations that are usually observed from one in vitro experiment to another.

The results show that the response of H spleen cells were consistently and markedly higher than that of L spleen cells. The maximal interline difference was obtained on the 4th day of culture.

For the whole range of antigen doses investigated, the number of PFC was about 100-fold higher in H than in L spleen cell cultures (Fig. 21). This represents an interline difference approaching that observed after in vivo immunization. These results indicate that the genetic difference between H and L responders concerns the immunocompetent cells themselves, thus excluding the intervention of hormonal or any other mechanism of immune regulation originating from the rest of the organism.

The immunocompetent cells belong to two cell types: lymphocytes and macrophages, both required to obtain an in vitro immune response, as shown in Table 14.

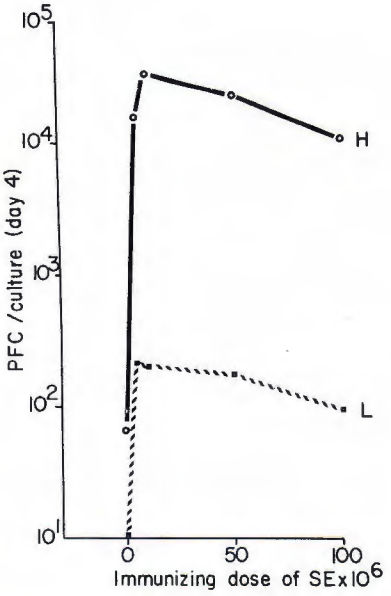

Fig. 21. In vitro responses of unseparated spleen cells cultures of H and L lines from Selection I (Mishell and Dutton culture system). PFC/culture on the 4th day post immunization with increasing doses of SE. (From *Doria* et al., 1978)

Table 14. In vitro anti SE antibody synthesis by 10^7 H or L (Selection I) spleen cells immunized in vitro with 10^7 SE

Cells in culture	ME[a]	PFC/culture (day 4)[b]	
		H	L
Unseparated	−	33000	210
	+	29800	2085
Non-adherent[c]	−	260	0
	+	24300	760

From *Doria* et al., 1978.
[a] ME added in the culture at a final concentration of 5×10^{-5}.
[b] PFC = Plaque forming cells on the 4th day of Mishell and Dutton culture.
[c] Separated by the method described by *Mosier* (1967).

Table 15. In vitro anti SE antibody synthesis by recombined H or L macrophages (1.5×10^7) and lymphocytes (10^7)

Lymphocytes versus macrophages					Macrophages versus lymphocytes				
Lymph	+	Macroph	PFC[a]	Ratio	Macroph	+	Lymph	PFC	Ratio
H		H	29700	11.2	H		H	29700	2.4
L		H	2640		L		H	12200	
H		L	12200	25.2	H		L	2640	5.4
L		L	484		L		L	484	
H		−	3110	222	H		−	4190	72
L		−	14		L		−	58	

From *Doria* et al., 1978.
PFC/culture of unseparated cells (1.5×10^7): H = 22600; L = 60.
[a] PFC: Plaque forming cells on the 4th day of Mishell and Dutton culture (immunization 10^7 SE).

The removal of macrophages, by plastic adherence (*Mosier*, 1967), suppressed, in both lines, the immune response of spleen cell populations.

The addition of ME to the cultures somehow replaced the macrophage function, restoring responsiveness in these depleted cell populations.

An attempt to measure, in H and L lines, the respective importance of lymphocytes and macrophages in the production of in vitro immune response was made by cell separation and recombination between the two lines. The results are summarized in Table 15.

As previously reported, the response of non-adherent cells was markedly reduced in comparison with that of mixed populations of both cell types. The small response obtained in separated cell cultures of H line only, resulted from a residual contamination of one cell type by the other.

The role of lymphocytes versus macrophages was measured by recombining H and L macrophages with either H or L lymphocytes. The difference in PFC attributable to lymphocyte origin was about 18-fold (11–25). The role of macro-

phages versus lymphocytes was evaluated by recombining H and L lymphocytes with either H or L macrophages, the difference in PFC attributable to macrophage origin was about 4-fold (2.4–5.4).

The results of cell recombination experiments indicate that the genetic difference between H and L responders is phenotypically expressed at the level of both lymphocytes and macrophages. The role played by lymphocytes in the in vitro immune response to SE was 4- or 5-fold more important than that played by macrophages. These results are consistent with those obtained in vivo (Paragraph A and B).

The non-specific effect of Selection I on the antibody response to other antigens (Chapter IV) was also demonstrated in in vitro experiments.

A 10-fold difference in favour of H line was observed in the in vitro response to trinitrophenyl (TNP) coupled with the T independent lipopolysaccharide from E. coli. These results, as well as those obtained in vivo with the Pneumococcus polysaccharide SIII, another T independent antigen, point out the genetic modification of B lymphocytes.

The helper effect of T cells of H and L lines was studied in vitro by adding an increasing number of in vivo carrier primed irradiated cells to F_1 spleen cells, and then by measuring the response to the hapten after in vitro immunization. The results showed that spleen cells from H or L mice displayed the same helper effect on the immune response of carrier primed cells in vitro. These experiments, described in detail by *Doria* et al. (1978) indicate that the genetic modification at the level of lymphocytes does not affect the helper function of T cells. A possible effect on the suppressor T cell function remains to be investigated.

E. Dissociation Between the Genetic Control of Humoral and Cell Mediated Immune Response

Perhaps the most important finding arising from the results of Selection I is that H and L lines, which differ so much in their general antibody responsiveness, have a similar ability for T cell mediated immunity.

The best demonstration of this fact was given by experiments of skin graft exchange between the two lines. Skin graft rejection is essentially a T cell mediated phenomenon.

There is a strong histoincompatibility between H and L mice since they differ at the H-2 locus (Chapter III.A.3). Skin grafts exchanged between the two lines are rapidly rejected. The mean rejection time of H skin grafts by L recipients was 10.4 ± 2.4 days and that of L skin grafts by H recipients was 12.6 ± 2.9 days. After skin graft rejection the serum titre of anti H-2 cytotoxic antibody was 1/110 in H line recipients and 1/3 in L line recipients. In fact, the mean survival time of skin allografts was significantly shorter in L than in H recipients ($p < 0.01$). This may be due either to the effect of facilitating antibody produced in larger amount in H line, or to a stronger participation of the very active macrophages of L line in the mechanism of skin graft rejection (*Liacopoulos-Briot* et al., 1972).

The selective breeding for SE responsiveness has therefore greatly modified the antibody response to histocompatibility antigens without changing the cellular immunity responsible for skin graft rejection.

Other T cell mediated reactions such as graft versus host reaction (*Byfield* and *Howard*, 1972), skin delayed hypersensitivity (*Mouton* et al., 1974), and in vitro response of T lymphocytes to phytohemagglutinin (*Liacopoulos-Briot* et al., 1974) have the same intensity in H and L mice.

All these findings converge in the clear demonstration that the genes regulating quantitative antibody response do not operate on cell mediated immunity. Antigen handling by macrophages seems to be required for induction of both antibody response and cell mediated immunity. The dissociation of these two phenomena in H and L lines may be explained according to two hypotheses. The pathway of antigen metabolism is different for antibody production and for cell mediated response (qualitative hypothesis). The quantitative hypothesis seems more probable. It is well known that the amount of antigen required for induction of cellular immunity is markedly smaller than that required for antibody response. A sufficient antigenic stimulation would be provided even by L line macrophages for the induction of cell mediated immunity. The shortage of immunogenic stimulation would therefore, in L mice, be a limiting factor for antibody response only.

In the literature several reports, mentioned later, indicate that cell mediated immunity is also submitted to genetic control. We may therefore conclude that the two fundamental functions of specific immunity: humoral and cellular functions, have an independent genetic regulation. This finding, together with the inverse relationship between antibody response and macrophage activity, demonstrated in a preceding chapter, constitutes the bases for the theory proposed to explain the multidirectional efficiency of the immunity considered as a defense system at the level of a genetically polymorph population (Chapter VI).

VI. Relationship Between Genetic Regulation of Immunoresponsiveness and Resistance to Aggression

As mentioned in the introduction, defence against aggression is based on the non-specific immunity mediated by macrophages and other phagocytic cells, and the specific cellular and humoral immunity mediated respectively by T and B cells. The two specific functions keep the memory of preceding stimulations. Therefore they chiefly intervene in the increased resistance induced by antecedent fortuitous (endemic) or intentionally induced (vaccination) contacts with pathogens.

Each type of infections is characterized by a peculiar aggressive device which is more efficiently contented by one or the other of the three immunity functions.

The results obtained in Selection I clearly demonstrate that each immunity function is submitted to an independent genetic regulation. The selective breeding greatly modifies the antibody responsiveness without changing the cell mediated immunity. Moreover an inverse relationship is observed between macrophage

activity and antibody responsiveness. Individuals genetically endowed with active macrophages are poor antibody responders and vice-versa. This inverse relationship is produced by the genetic modification of enzymatic antigen handling inside the macrophages of H and L antibody responders. This modification is responsible for the different bactericidal activity of macrophages when living micro-organisms are phagocytized. An individual with active macrophages will survive an infection to which the resistance is phagocytosis dependent, but will be killed by an infection efficiently coped with by antibodies and vice-versa.

The third defence mechanism is constituted by the cell mediated immunity. This response is also submitted to genetic control (*Chase,* 1941; *Benacerraf* and *McDevitt,* 1972; *Miller* et al., 1976). A major histocompatibility complex linked Ir gene is of paramount importance in this control but other independent genes also intervene in the quantitative regulation of T cell mediated responses (*Shultz* and *Bailey,* 1975; *Lubet* and *Kettman,* 1978). It is therefore very probable that cell mediated immunity to complex immunogens such as bacteria and viruses is submitted to quantitative polygenic regulation. The phenotypes of this character in a genetically heterogeneous population will therefore present a normal frequency distribution. A recent and very interesting report by *Fachet* and *Ando* (1978) demonstrates an inverse quantitative regulation of cell mediated and humoral responses analogous to our findings concerning macrophage activity and antibody responsiveness (Chapter V.B).

The independent and polygenic regulation of the three fundamental immunity functions constitutes the genetic arrangement providing the optimal defence of a genetically polymorphic population against all types of mild endemic and severe epidemic infections. In fact the largest number of individuals whose phenotypes are close to the mode of each immunity parameter will have the best multidirectional defence against all endemic infections. Therefore the genetic polymorphism of the population will be maintained by stabilizing natural selection. When the population is threatened by a severe epidemic burst the extreme phenotypes of the relevant immunity parameter will survive. As a consequence a number of individuals, inversely proportional to the epidemic severity, will resist each type of epidemic, thus ensuring the population survival.

The natural history of a population results from its confrontation with different types of epidemic infections that are coped with by one or the other of the immunity functions. Therefore directional natural selection will not appreciably modify the modal phenotype distribution resulting from polymorphism unless the population is submitted to recurrent epidemic infections of the same type.

These considerations may be applied to both natural resistance to infections and protective effect of vaccination.

The essential immunologic characteristics of H and L lines of mice of Selection I, schematized in Table 16, permit the identification of the mechanism responsible for natural or vaccination induced resistance against the different types of infection.

When untreated or vaccinated H and L mice are challenged with various infections, three outcomes may be anticipated according to the type of anti-infection defence implicated.

Table 16. Schematic representation of the immune characteristics of H and L mice of Selection I

	Humoral immunity response	Cellular immunity response	Microbicidal effect of macrophages
H line	+++	++	+
L line	+	++	+++

1. If antibodies play an important role, H mice are expected to be more resistant than L mice.

2. If macrophage activity is determinant, L mice are expected to be more resistant than H mice.

3. If cell mediated immunity plays the essential defensive role, equal resistance is likely to be found in both lines.

This last outcome may be occasionally masked by a higher production of facilitating antibody in H line. This possibility can be experimentally tested by passive immunization of L recipients with H line immuneserum.

Of course, cooperation or antagonism may exist between the three immunity mechanisms. The final issue resulting from their balance will depend on the limiting parameter.

The three possible outcomes have been verified in different types of infection (*Biozzi* et al., 1978). Some typical results are now briefly presented.

A. Anti-Infection Immunity

1. Macrophage Dependent Immunity

Gram negative micro-organisms such as Salmonella, Brucella, Yersinia, Mycobacteria, are "intracellular parasites". They are easily phagocytized by the macrophages but can survive and multiply inside the phagocytic cells. The antibodies are inefficient against this type of infection.

The specific or non-specific stimulation of macrophages activity produces an efficient protection against Salmonella infections (*Biozzi* et al., 1957; *Howard* et al., 1959; *Mackaness* and *Blanden*, 1967).

The results of several experiments on the resistance of H and L mice against Salm. tm. infection are summarized in Table 17 (*Biozzi*, 1972). As expected the antibody response to both flagellar and somatic antigen of Salm. tm. was much stronger in H than in L mice (Fig. 17). On the contrary, the natural resistance to this pathogen was stronger in L than in H mice. This difference was detectable in terms of mean survival time when an extremely severe infection was produced by intraperitoneal challenge. When the severity of the infection was decreased by subcutaneous challenge, the stronger resistance of L line was evident in terms of definitive survival. Both low virulence Salm. tm. vaccination and BCG pretreatment allowed a 90%–100% survival of L mice against a 100% lethal infection in controls. No survival was observed in pretreated

Table 17. Natural resistance and protective effect of BCG and of vaccination against Salm. tm. infection in H and L lines

	Challenge Number of Salm. tm. injected	H line		L line	
		per cent mortality	Mean survival time (days)	per cent mortality	Mean survival time (days)
Control	1000 I.P.	100	5.4	100	8.7
Natural resistance	5000 S.C.	100	10.4	45	—
Specific vaccination with living low virulence Salm. tm.	1000 I.P.	100	8.6	10	—
Non-specific protection[a] with living M. tuberculosis BCG strain 14 days previously	1000 I.P.	100	11.9	0	—

From *Dodin* et al., 1972.
From *Biozzi* et al., 1972c.
[a] BCG 4×10^6 viable units i.v.

H mice which showed only an increase of the mean survival time compared to the controls. Thus both natural and induced resistance to Salm. tm. infection were stronger in L antibody responders endowed with very active macrophages.

Identical results were obtained in the severe infection induced by subcutaneous inoculation of 1000 highly virulent Yersinia pestis. This severe challenge produced a 100% mortality in both lines. The stronger natural resistance of L mice was demonstrated by a mean survival time of 7.7 days compared to that of H mice (4.5 days). This interline difference was markedly amplified after vaccination with 500 μg of protective Y. pestis extract. No protection was produced in H mice which all died within a mean survival time of 5 days, while 100% of the vaccinated L mice survived the infection (*Dodin* et al., 1972).

A study of Brucella suis (B. suis) infection in H and L mice was carried out by *Cannat* et al. (1978). The antibody response to B. suis was 16-fold higher in H than in L mice. As predictable by the results of Chapter V, the delayed cutaneous hypersensitivity to melitin was of similar intensity in the two lines. Both natural and post-vaccinal resistances to B. suis infection were stronger in L than in H mice. In these experiments the resistance to infection was measured by the number of surviving B. suis inside spleen macrophages. The bactericidal activity of macrophages constitutes the principal defence against B. suis infection while antibodies only play an accessory role.

Howard (personal communication) studied, in H and L mice of Selection I, the natural resistance to Leishmania tropica (L. tropica), a typical parasite of macrophages. Although the antibody response to L. tropica antigens was much higher in H than in L line, the resistance against this infection was stronger in L line. The subcutaneous injection of this parasite produced in L mice small and transient local skin lesions which healed within 1–2 months

and all the mice survived. In contrast, in H mice the local lesions were extremely large, causing a high percentage of mortality. The comparison of L. tropica survival inside the peritoneal macrophages of H and L mice confirmed the primordial importance of these cells for the natural resistance against this infection.

All the above-mentioned examples demonstrate the higher resistance of L mice against "intracellular parasite" infections.

2. Antibody Dependent Immunity

An antibody dependent host defence mechanism operates in Trypanosoma and Plasmodia infections.

Trypanosoma cruzi (T. cruzi) infection was studied in H and L mice by *Kierszenbaum* and *Howard* (1976). The antibody titre to T. cruzi antigens, after injection of a sublethal dose of parasite was 1/945 in H mice and $<1/20$ in L mice. The natural resistance to this infection was markedly higher in H than in L mice. The dose of living parasites inducing a 50% mortality (LD 50) was 270–1 000 times larger in H than in L mice.

An active specific vaccination failed to protect L mice while an efficient protection was produced in H mice. It was clearly demonstrated that the inefficacy of vaccination in L mice was due to their genetic defects in antibody response since protection could be induced in these mice by passive administration of immune serum from vaccinated H mice.

Antibody dependent post-vaccinal immunity was also demonstrated in Plasmodium berghei (P. berghei) infection as shown in Table 18.

The similar percentage of mortality and survival time in H and L infected controls indicate that the natural resistance against P. berghei infection is independent from both antibody response and macrophage activity.

The protective effect of vaccination, on the contrary, is much stronger in H than in L mice. The antibody titre to P. berghei measured by immunofluorescence in vaccinated mice was 1/11 000 in H mice and 1/1 000 in L mice. Similar results have been obtained in H and L mice of Selection II. The concordance

Table 18. Natural resistance and protective effect of specific vaccination in Plasmodium Berghei infection

	H line		L line	
	Mortality	Mean survival time (days)	Mortality	Mean survival time (days)
Control mice	84%	16.6	95%	17.7
Vaccinated mice	5%	–	85%	19

Biozzi et al., 1978.
Infection: 10^7 i.p. parasitized mouse erythrocytes.
Vaccination: 6 i.p. injections a week apart from 3×10^7 parasitized mouse erythrocytes irradiated with 60 000 r.
(This investigation received financial support from the World Health Organization)

of results in Selections I and II substanciates the importance of antibody response in the mechanism of post vaccinal immunity. Moreover, in Selection II, a positive correlation ($r=0.88$) was found between the antibody titre in the vaccinated mice and the percentage of survival to P. berghei infection in H and L lines and their interline hybrids F_1, BcH and BcL.

Recently, *Nilsson* et al. (in press) investigated the antibody response to rabies virus and the protective effect of vaccination in H and L mice of Selections III and IV. The antibody titre measured by virus neutralization test and the resistance to infection induced by intracerebral challenge were determined. In Selection III the antibody titre was about 10-fold higher in H than in L mice; this difference was smaller in Selection IV. The natural resistance of non-vaccinated mice was similar in H and L lines of both Selections. The protective effect of vaccination was stronger in the H lines than in the L lines of the two Selections. This effect was more pronounced in Selection III than in Selection IV. The efficacy of vaccination was roughly related to the level of antibody response of the four lines of mice.

B. Anti-Helminthic Immunity

Immunity against large multicellular parasites such as Schistosoma mansoni (S. mansoni) (*Blum* and *Cioli*, 1978), and Trichinella spiralis (T. spiralis) (*Perrudet-Badoux* et al., 1975, 1978) was investigated in H and L lines of Selection I. The antibody response to antigens extracted from these two parasites was always markedly higher in H mice.

The innate resistance to S. mansoni infection was stronger in L than in H mice, moreover L mice developed an acquired resistance to re-infection as good and even better than that of H mice. In this infection therefore, antibodies do not seem to play the efficient protective role which is rather to be attributed to macrophages (*Blum* and *Cioli*, 1978).

The IgG_1 and IgE antibody titre to T. spiralis antigens was 25- to 50-fold higher in H than in L mice (*Perrudet-Badoux* et al., 1975). The level of natural resistance to primary infection produced by a low number of larvae (50) was higher in H line, nevertheless L mice acquired a total protection against re-infection while only a partial protection was acquired by H mice. These results suggest that immunity to primary or secondary T. spiralis infection depends on different mechanisms (*Perrudet-Badoux* et al., 1978).

C. Anti-Tumoral Immunity

1. Allogeneic Tumors

Two allogeneic tumors were transplanted in H and L mice: the Ehrlich ascitic carcinoma and the Sarcoma 180.

The susceptibility to Ehrlich carcinoma was similar in H and L lines: a 100% mortality and an equivalent survival time was observed in both lines.

In contrast, a striking superiority of L line resistance against Sarcoma 180 was demonstrated. This tumor grew rapidly in H line, inducing a 90% mortality within 50 days. In L line, on the contrary, after an initial growth the tumor regressed and disappeared in all the mice. This striking difference between the results obtained in Ehrlich carcinoma and in Sarcoma 180 is very probably due to the high sensitivity of Sarcoma 180 to the enhancing effect of antibody whilst the Ehrlich tumor is resistant to enhancement (*Biozzi* et al., 1972c). Some unpublished experiments of transfer to L mice, of serum from H mice bearing tumors, suggest that the growth of Sarcoma 180 in L line was suppressed by lack of enhancing antibody response. The possible contribution of the active macrophages of L mice in tumor immunity is not excluded.

2. Syngeneic Tumors

The syngeneic DBA/2 and AKR leukemia, the C_3H mammary carcinoma and strain XVII lymphosarcoma were studied. These tumors only grow in the syngeneic strain, not in H nor L mice. Therefore the study was carried out in F_1 hybrids between the syngeneic strains and H or L mice. It was previously demonstrated that these F_1 hybrids retain a part of the parental interline difference in antibody responsiveness characterizing H and L lines. The tumor induced mortality and the rate of tumor growth was similar in H and L F_1 hybrids (*Biozzi* et al., 1972c). According to the model presented in Table 16, if immune resistance were induced during the growth of these tumors, it should be due to cell mediated immunity without decisive participation of either macrophage activity or antibody response.

3. Carcinogen Induced Tumors

The tumors were induced in mice of Selection I and Selection II by intramuscular injection of 200 μg of 3–4 benzopyrene in oil solution. In both Selections the tumor incidence was higher in L than in H line. Nine months after carcinogen injection the tumor incidence was 13% in H line and 52% in L line of Selection I (*Biozzi* et al., 1972c). In Selection II the tumor incidence was 35% in H line and 54% in L line. The interline difference in tumor incidence was larger in Selection I than in Selection II, this may be due to the difference in the non-specific effect of the two Selections on responsiveness to unrelated antigens which is smaller in Selection II than in Selection I (Chapter IV).

The incidence of 3–4 benzopyrene induced tumors was investigated in interline hybrids: F_1, BcH and BcL of Selection I, and compared with the antibody response to a threshold dose of EA. This antigen was chosen since the supposed tumor antigens are unrelated to the selection antigens. The choice of the threshold dose is justified since it is generally admitted that tumor antigens are present in minute amounts in the initial stage of tumor development when anti-tumor immune defence is likely to be effective.

The results of this experiment, reported in Fig. 22, show a high correlation ($r=0.91$) between the antibody response and the anti-tumor resistance measured

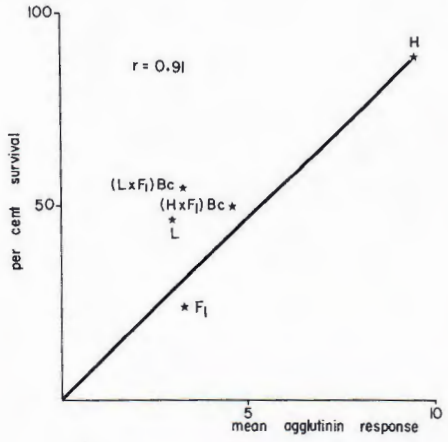

Fig. 22. Correlation between resistance to carcinogen induced tumours and responsiveness to threshold dose of unrelated antigen in H and L lines of Selection I and their interline hybrids. Per cent survival in groups of 30–36 mice 9 months after intra-muscular injection of 200 μg 3.4 benzopyrene plotted against the mean of maximal agglutinin titres after i.p. injection of 0.05 μg EA in groups of 10–20 mice (correlation calculated by a least square regression curve)

by the survival percentage. These findings do not demonstrate but suggest a possible role of antibody responsiveness in the resistance to carcinogen induced tumors. In this respect it should be remembered that antibody responsiveness to the threshold dose of EA is a recessive character in F_1 hybrids and is controlled by a single H-2 linked locus (Chapter IV.A.). The importance of H-2 linked genes in the anti-tumor immunity is an open possibility while their intervention in anti-infection immunity is unlikely, as hypothesized in the introduction.

4. Incidence of Spontaneous Tumors

This study was performed by *Covelli* et al. (1978) in H and L mice of Selection I. The mice were kept in the same animal department until spontaneous death. They were then submitted to systemic macroscopic and histologic examination.

The first finding was that L mice had a shorter life expectancy than H mice. The mean life span was about 450 days in L line and 700 days in H line. This interline difference in longevity was precedently observed by Howard in colonies of H and L mice bred in his animal department (personal communication).

The observations of neoplastic pathology by *Covelli* et al. demonstrated that the incidence of malignant tumors was 4% in H mice and 32% in L mice ($p < 0.025$). The majority of tumors were generalized lymphomas and lung invasive adenocarcinomas sometimes associated with metastasis. The occurrence of lymphomas was about 10-fold more frequent in L than in H mice ($p < 0.05$). This interline difference in spontaneous tumor incidence is large enough to have a significant impact on longevity.

This study, like the preceding one on the carcinogen induced tumors, does not demonstrate, but clearly suggests a possible causal relation between antibody responsiveness and anti-tumor immunity.

VII. Summary

The study of the genetic regulation of immune response to natural multideterminant immunogens was undertaken by the method of bidirectional selective breeding of High or Low antibody responder lines of mice. Five Selections are described:

Selection I, carried out for agglutinin responsiveness to sheep erythrocytes and pigeon erythrocytes alternated in each generation.

Selection II, carried out for agglutinin responsiveness to sheep erythrocytes repeated in each generation.

Selection III and Selection IV performed respectively for agglutinin response to flagellar or somatic antigens of Salmonella typhimurium and Salmonella oranienburg alternated in each generation.

Selection V, performed for passive agglutinin response to bovine serum albumin and rabbit gamma globulin alternated in each generation.

In each Selection the character investigated is polygenic. High and Low responder lines diverge progressively during the selective breeding. The maximal interline separation (selection limit) is reached in the 7th–16th generations. High and Low responder lines at selection limit are considered homozygous for the character submitted to selection. Their variance is therefore only due to environmental effects.

The difference in agglutinin titre between High and Low lines is 220-fold in Selection I, 103-fold in Selection II, 90-fold in Selection III, 85-fold in Selection IV and 275-fold in Selection V.

The partition of genetic and environmental variances in the foundation populations of the five Selections is established. The proportion of genetic variance is 60% in Selection I; 49% in Selection II; 51% in Selection III; 47% in Selection IV and 76% in Selection V.

The heritability of the character investigated is about 0.20 in the five Selections.

The approximate number of independent loci regulating the quantitative antibody response is between 9–15 in Selection I, 2–12 in Selection II, 7–18 in Selection III, 5–12 in Selection IV and 2–4 in Selection V.

The effect of the polygenic regulation of responsiveness to selection antigens is essentially non-specific. The same group of genes regulates the antibody response to many complex immunogens unrelated with those used during the selective breeding. The extent of this non-specific effect depends on the nature of the antigens and on the immunization procedure. It is very large in Selections I and III, intermediate in Selections II and IV, and restricted in Selection V.

The immunobiologic effect of the genes regulating antibody responsiveness was extensively studied in Selection I. The principal results are:

1. The selective breeding only affects antibody responsiveness. The cell mediated immune responses have the same intensity in High and Low antibody responder lines.

2. The metabolic and bactericidal activities of macrophages are markedly higher in Low than in High antibody responder line.

The natural resistance against various experimental infections and tumors, and the protective efficiency of vaccination were studied. The genetic independence of the immunity functions permits the identification of the limiting factor responsible for the anti-infection immunity.

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p68 even SRBC ind'd responses - SEL'N → all sorts of xgen specs

p. 78 LARGE # OF LOCI ~ BROAD SPECIFICITY OF H-L DIFF

p87 MΦ - xB tradeoff b/c of a genic 1/2 locus?

STAB'G SEL'N - INTERMED IS OPTIMAL

BAL'G " HI OPTIMAL IN SOME CONTEXTS

LO " " OTHERS