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EDITORIAL

Correlation between genetic regulation of immune responsiveness and host defence against infections and tumours

The teleonomic function of the immune system is the defence of the host against infections by viruses, bacteria or parasites. In view of this protective role, the efficacy of the immune system was undoubtedly a character of high selective value during evolution, in natural genetically heterogeneous populations.

The anti-infectious immunity is essentially based upon three mechanisms: phagocytosis and intracellular killing of pathogens by macrophages, cell mediated immunity and humoral immunity. The phagocytosis is an extremely old mechanism already present in amoebae, then the cell mediated immunity emerged in invertebrates and finally the antibody synthesis appeared in primitive fish. These last two mechanisms are characterized by two new and very important properties: specificity and memory. Successively acquired, these three functions cooperate in perfect coordination and have remained unchanged for hundreds of millions of years: from fish to Man. Such a remarkable invariance is in fact mainly explained by the excellent performance of the immune system in protecting animal populations against all types of infections. In the course of our study on genetic quantitative regulation of immune functions in the mouse, we obtained results which correlate to this view and support the proposal of a theory accounting for the efficacy and, consequently, the stability of the immune system.

Our experimental approach of the study of the genetic control of immune responsiveness was to carry out selective breedings of mice for high or low immune responses; in other words, to realize artificial disrupting selections. This lecture will only deal with the first selection experiment performed for antibody responsiveness (Selection I) [1]. This selection is actually the most extensively studied, and the most relevant for the topic of this lecture.

Description of the selection experiment

The phenotypic character chosen for the selection was the maximal antibody titre produced in outbred individual mice immunized with an optimal dose of complex immunogens: heterologous erythrocytes. A normal continuous distribution of phenotypes is observed in the genetically heterogeneous foundation population, when antibody titres are expressed as ln of agglutinin titres. The selective breeding was based on the assortative mating of individuals with extreme phenotypes, repeated through successive generations. It produced the progressive divergence of a 'high' (H) and a 'low' (L) antibody responder line of mice. When the interline difference reaches its maximal value (sixteenth generation) the mice of each line can be considered as homozygous at all the loci involved in the regulation of the character submitted to selection. The mean antibody response is then 220-fold higher in H than in L mice [1, 2].

The analysis of the antibody responses in each line during the selective breeding and in hybrids between H and L homozygous mice gave the following results:

(i) The variability of antibody responses in the foundation population and in F_2 segregant hybrids results from both environmental effects and genetic factors, each responsible for about 50% of the total phenotypic variance.

(ii) The interline difference is due to the additive effect of about ten independent loci, supposed to have equivalent effects. This assumption was verified for two identified loci: the H-2 and the Ig allotype.

(iii) The heritability value of the character in this selection experiment was 0·20 [3].

Cellular expression of the genes regulating high and low antibody responsiveness in H and L mice

Since the character submitted to selection was the serum antibody titre, i.e. the final step of the complex cell interaction produced by immunization, the effect of selection can be expected at every stage of antibody production.

A cytodynamic study of antibody producing cells was carried out in H and L lines [2]. Both *in vivo* and *in vitro* experiments have shown that the potentialities of B lymphocytes clearly differ in H and L mice. In contrast, no interline difference was found at the level of T lymphocyte activities as far as T mediated immune reactions are concerned: time of allogeneic graft rejection, intensity of delayed type hypersensitivity reactions and of graft versus host reactions, and lymphoproliferative responses to phytohaemagglu-

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tinin, are similar in both lines. These important findings demonstrate that T mediated immunity and antibody production are under distinct genetic controls.

An important effect of the selection concerns the macrophages. The differences observed in the macrophage activity between H and L mice strongly modify their degree of resistance against some pathogens. Therefore, they will be described in a more detailed way. The phagocytic activity of macrophages of the reticulo-endothelial system is quite similar in the two lines but in contrast there is a sharp difference between H and L mice for macrophage activities related with their enzymatic equipment. It has been shown that the rate of antigen catabolism is much more rapid in L than in H macrophages. As an example, the immunogenic fraction of sheep erythrocytes persists longer in the spleens of H than in the spleens of L mice (10 days instead of 4 days). This difference stresses the major regulatory role of macrophages in antibody production. The rapid breakdown of antigen in macrophages of L mice provokes an antigen shortage which is mainly responsible for the poor antibody response in these mice. In contrast, prolonged antigenic stimulation permits a sustained antibody response in H mice.

The role of macrophage modification in interline difference explains also an important finding in Selection I: the non-specific effect of the selection. In fact, it has been constantly observed that the effect of the genes responsible for high or low antibody responses in the two selected lines is not restricted to the antigens used during the selective breeding. A similar interline difference is obtained when H and L mice are immunized with antigens of different structures and specificities: proteins, haptens, bacteria, parasitic antigens, transplantation antigens ... Moreover, the interline difference includes the production of all classes of immunoglobulins.

In parallel with the modification of antigen catabolism, the selection has also affected the ability of macrophages of H and L mice to control the intracellular multiplication of engulfed pathogens. The macrophages of L mice are much more efficient than H mice macrophages in this respect and this was observed towards various micro-organisms, as we shall see later on. First it is worth emphasizing that the genes accumulated in H and L mice have a completely opposite effect on two mechanisms of anti infectious resistance: H mice which are able to produce very high antibody titres have a weak capacity to control the multiplication of pathogens inside macrophages. On the other hand, L mice, in which only poor antibody responses are obtained, have a great advantage at the level of macrophage bactericidal/bacteriostatic power.

The main characteristics of H and L mice described above can be summarized as follows:

	Antibody production	Macrophage activity Antigen catabolism Bacteriostatic power	T cell mediated immunity reactions
H line	+++	+	++
L line	+	+++	++

These clear-cut differences between H and L mice are expected to have important consequences on their resistance to infections.

Resistance of H and L mice to various infections

The capacity of resistance of H and L mice against various pathogens depends on the nature of the mechanism which plays the major role in the host defence against each pathogen. In a first group of infections, the presence of antibodies is recognized as an important factor of resistance. This is the case of infections by cocci (pneumococcus, streptococcus) and in such situations a clear advantage of H mice can be anticipated, while L mice will be particularly susceptible. The resistance of H mice should also increase after specific vaccination.

A major protective role of antibodies is also observed in certain parasitic diseases. Interesting results have been obtained in H and L mice challenged with various parasites. *Plasmodium berghei* is the agent of malaria in the mouse. It provokes a severe disease, and a 100% mortality occurs within a few days in the two lines. However, when H and L mice are infected with *P. berghei* after an appropriate specific vaccination schedule, a very efficient protection is obtained in H mice (together with high anti parasite antibody titres) since most of these mice survive the infection whereas the vaccination has no effect in L mice in which only low antibody titres are found [4].

Analogous results were observed in the course of infection with *Trypanosoma cruzi*. An interline difference in resistance in favour of H mice is already noticed in normal mice (natural resistance). As before, the specific active immunization against the parasite induces in H mice a high antibody titre associated with an enhancement in resistance. This does not occur in L mice, but these mice can be efficiently protected against *T. cruzi* challenge by the passive administration of specific antibodies [5]. This result clearly points out the role of antibodies in the resistance against the parasite.

Antibody plays a protective role also in metazoal infection by large parasites such as intestinal worms. H mice were shown to be more resistant than L mice against the worm *Nematosporoides dubius* [6]: the enhancement of resistance is clearly observed, in H mice only, when reinfection is given for a second time after the first infection has been cured.

The degree of resistance of H and L mice was also investigated against infections of another type. These infections have a common aetiology since they are provoked by intracellular parasites, i.e. by bacteria or parasites which are able to survive and multiply inside the macrophages. Against this type of infections, therefore, the host resistance essentially depends on the non-specific bacteriostatic power of macrophages. A striking difference was observed in the capacity of H and L mice to cope with this kind of infections and, in

contrast with the above reported results, L mice were much more resistant than H mice.

This was first demonstrated during the course of infection by *Salmonella typhimurium*. A high degree of resistance is observed in normal L mice whereas H mice are extremely susceptible to the infection. There is a more than 100,000 times interline difference in LD_{50} after subcutaneous challenge [7]. The mortality rates are correlated with the efficacy of the control of *S. typhimurium* multiplication which is very strong in L mice while it is lacking in H mice. If the intravenous route is used for infection, a more severe disease occurs and all mice of the two lines die. However, the survival time is still much longer in L than in H mice. The more remarkable result is the fact that vaccination can protect very efficiently L mice against the severe intravenous challenge whereas no effect is obtained in H mice [8]. These data are in agreement with the interpretation that vaccination protects mice through a stimulation of macrophage activity while antibodies do not play a role in the early defence against *S. typhimurium*.

A clear-cut advantage of L mice at the level of both innate and acquired resistance is also observed during the course of *Yersinia pestis* infection. Furthermore, a strong bacteriostatic/bactericidal power of L mice macrophages was demonstrated towards *Brucella suis* [9] and Mycobacteria (BCG) [10].

Finally, L mice proved to be more resistant than H mice against the infection provoked by the parasite *Leishmania tropica* [11]. The cutaneous lesions due to the intracellular multiplication of the parasite increase and ulcerate in H mice among which an appreciable percentage of mortality occurs. In contrast, the initial lesions heal rapidly in L mice which survive the infection.

In conclusion, these results demonstrate that the outcome of infectious diseases in H and L mice is closely related to their immune characteristics. H mice do have a huge advantage to cope with infections against which antibodies play an important defensive role but, in contrast, they proved to be highly susceptible to infections due to intracellular parasites. The exactly reversed pattern is observed in L mice.

Mechanism of anti infectious defence in natural populations

H and L mice can be considered as the extreme phenotypes which could be found in a natural population, which is similar to an interline F_2 hybrid population. The frequency of individual phenotypes for polygenic traits, in such populations, is expected to have a normal continuous distribution: most individuals being close to mean values whereas few individuals have extreme high or low values.

According to the inverse correlation demonstrated in Selection I, these individuals with extreme values would have opposite capacities for antibody production and for macrophage activity. Therefore most individuals, endowed with intermediate potentialities for these two functions, will be reasonably well protected against endemic infections whatever the defensive mechanism may be. In contrast, individuals from each distribution tail will be extremely susceptible to those mild infections which are counteracted by the mechanism for which they are genetically defective. Thus, the extreme phenotypes, on both sides of the distribution, will be constantly eliminated from the population. This produces a stabilizing selection which maintains the polymorphism at all the relevant loci. On the other hand, if any severe epidemic occurs, a small fraction of the population will be able to survive owing to a very efficient defence ensured either by antibody production or by macrophage activity. In spite of a high mortality rate, the population survives. Successive epidemics of the same type of infections could lead to a progressive change in the genetic constitution of the population. This does not occur if the successive pathogens threatening the population gives the advantage alternately to one or the other of the distribution tails.

The resistance due to T mediated immune reactions is not taken into account in this simplified hypothesis. Since T mediated immunity is under a distinct polygenic regulation, it may also contribute to maintain the genetic diversity.

This theoretical view strongly argues for the efficacy of the immune system for protecting natural populations against infections. On the other hand, the variety of the pathogens attacking the populations contributes to the maintenance of the polymorphism. The prolonged phylogenetic invariance of the immune system is due to the fact that it ensures an optimal protection against all types of infections at the level of natural populations.

Anti tumour resistance in H and L mice

The comments on resistance against infections apply to anti tumour defence mechanisms in so far as immune respone can be elicited against new antigens acquired by transformed cells. Several experiments have been carried out on resistance of H and L mice against tumours. The most relevant results concern the occurrence of spontaneous tumours and of carcinogen induced tumours since these conditions are closer to situations in human beings.

The incidence of naturally occurring tumours was checked in H and L mice kept until spontaneous death and then submitted to autopsy [12]. The percentage of neoplastic diseases was significantly higher in L than in H mice (32% versus 4%). The majority of tumours were generalized lymphomas and lung adenocarcinomas.

A similar interline difference was obtained when H and L mice were given a local injection of 3–4 benzopyrene. Nine months after injection, the tumour

incidence was 52% in L mice and only 13% in H mice. The rate of tumour growth was also lower in H mice [13]. These two findings could be explained by a protective role of antibody in the early phase of tumour development since H mice are able to mount an immune response to very low antigen doses.

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