GENETIC CONTROL OF MACROPHAGE FUNCTIONS

I. Polygenic Regulation of Phagocytosis Stimulation Produced by Glyceryl Trioleate

By DENISE MOUTON, YOLANDE BOUTHILLIER, NICOLE FEINGOLD, JOSUÉ FEINGOLD, CLAUDE DECREUSEFOND, CLAUDE STIFFEL, AND GUIDO BIOZZI

(From CNRS, INSERM, la Fondation Curie - Institut du Radium, 75231 Paris Cedex 05, France, and le Laboratoire d’Immunologie des Tumeurs, UER d’Hématologie, Hôpital Saint-Louis, Paris and l’Unité de Recherches de Génétique Médicale, Hôpital des Enfants Malades, Paris 15e)

Macrophages play an important role in various biological phenomena, such as immunoresponsiveness (1-3), nonspecific defence against infections or tumors (4-10), and lipid metabolism (11, 12). Macrophages are characterized by powerful phagocytic activity and the complex enzyme equipment of their lysosomes. These cells have been grouped by Aschoff and Kiyono in the reticuloendothelial system (RES).

The phagocytic activity of the group of RE macrophages, in contact with the circulating blood, can be measured from the rate of blood clearance of colloidal carbon particles injected intravenously (13, 14).

The majority (about 90%) of those macrophages are located in both liver and spleen. The basic phagocytic activity of this group of macrophages is fairly constant in different animal species when corrected by the relative mass of liver and spleen (15). Small variations of the phagocytic activity of RE macrophages have been observed in different inbred strains of mice (6, 16). Recent observations have demonstrated that these small variations are submitted to genetic control (17).

The phagocytic activity of RE macrophages can be strongly activated by administering different bacteria or bacterial products (4, 5, 10, 18-20), lipid emulsions (21, 22), and hormones (23, 24). Among bacteria, Mycobacterium tuberculosis (BCG strain) and certain corynebacteria (C. parvum) are the most active in stimulating RE macrophages. Responsiveness of macrophages to C. parvum and BCG is submitted to genetic control (20). Nevertheless, an attempt to separate “high” and “low” lines of mice for the character “macrophage responsiveness to BCG” was unsuccessful (16). On the contrary, it was possible to separate, by selective breeding, high and low responder lines of mice to the stimulatory effect of glyceryl trioleate (triolein). Preliminary results of this selective breeding experiment, carried out on eight consecutive generations, have already been reported (20).

Abbreviation used in this paper: h², heritability; R, response to selection; RES, reticuloendothelial system; S, selection differential; T, triolein.
The selective breeding, continued for 26 generations, produced a very marked interline separation of the character investigated. In the present article, a complete genetic analysis of the character “responsiveness of macrophages to stimulatory effect of triolein” is reported.

The phagocytic activity of RE macrophages was measured by the phagocytic index K after administration of triolein (T). Therefore, high and low responder mice were designated as KTH and KTL respectively. The responsiveness of RE macrophages to triolein is a polygenic character. The heritability (h²) of this character is rather low: about 12%. The genetic analysis indicates that the character investigated is determined by the cumulative action of about 27 independently segregating loci. The distribution of the character estimates among (KTH x KTL)F₁ and their backcrosses with both parental lines shows that low responsiveness is dominant.

**Materials and Methods**

*Selective Breeding.* The foundation population was composed of 25 male and 25 female adult random bred albino mice obtained from several commercial breeders. The selective breeding was made for the phenotypic character “macrophage responsiveness to triolein” measured by the phagocytic index K. Two lines of mice were separated: the high responders KTH and the low responders KTL. At each generation, 5-6 pairs of each line were selected out of a population including 20-40 animals. The mice were weaned when 30 days' old. The stimulation of RE macrophages by triolein was measured approximately 40-60 days after weaning. Brother-sister mating and interline crossing were excluded during the selective breeding.

*Genetic Analysis.*

**Estimation of Heritability.** The realized h² of the character was measured according to Falconer from the formula h² = R/S where R is the response to selection and S represents the selection differential (25).

**Calculation of the Number of Loci.** The number of independently segregating loci determining the character (n) was estimated as per the formula

\[ n = \frac{1}{8} \frac{R^2}{VA} \]

where VA is the additive variance. The additive variance is calculated from the phenotypic variance of the foundation population multiplied by the h². For a correct interpretation of the results, within the unavoidable limitations inherent in this method, see Falconer (25).

**Estimation of Dominance.** The global dominance of the character is expressed by the ratio: VD/VA where VA is the additive variance and VD is the dominance variance. This ratio can be calculated by comparing the F₁ values with the mean values of the parental lines according to the following formula, described in detail by Cavalli-Sforza and Bodmer (26):

\[ \frac{VD}{VA} = \frac{1}{2} \left( \frac{1}{2} (KTH + KTL) - F_1 \right)^2 \]

Due to the fluctuations of K in successive generations, the means of the values established in the 25th and 26th generations were used to express KTH and KTL values.

*Measure of the Phagocytic Activity of RE Macrophages.* The phagocytic index K was measured
GENETIC CONTROL OF MACROPHAGES

48 h after administration of triolein, from the rate of blood clearance of 8 mg/100 g of colloidal carbon injected intravenously (Colloidal carbon preparation 1431/a Gunther Wagner, Hanover, Germany). The phagocytic index K was calculated, as previously described, from the formula:

\[ K = \frac{\log_{10} C - \log_{10} C'}{t' - t} \]

where \( C \) and \( C' \) are the blood carbon concentration expressed in mg/100 ml of blood, respectively at the times \( t \) and \( t' \), expressed in minutes, after the intravenous injection of colloidal carbon (14). In some experiments, a more detailed study of the RE macrophages activity was performed by calculating the corrected phagocytic index \( \alpha = \sqrt{K (W/WLS)} \) (\( W \) is the body weight and \( WLS \) the weight of liver and spleen). The corrected phagocytic index \( \alpha \) expresses the macrophage activity for a constant weight of liver and spleen (13).

Preparation of Triolein Emulsion. 5 ml of pure glyceryl trioleate were added to 0.33 ml of Tween 20, then emulsified, using an ultraturax disperser, in 45 ml of 5% aqueous solution of glucose. The emulsion was prepared just before it was intravenously injected (0.2 ml of the emulsion per 20 g of body weight).

Results

Separation of KTH and KTL Lines. The basic phagocytic activity of RE macrophages, measured in a group of 30 untreated mice of both sexes, of same origin as the foundation population, gave the following values: \( k = 0.019 \pm 0.005 \), \( \alpha = 4.8 \pm 0.6 \); liver/20 g = 1.027 mg \( \pm \) 70, spleen/20 g = 90 mg \( \pm \) 30. No significant sex difference was found in the normal phagocytic activity of macrophages.

The maximal stimulation of macrophage phagocytic activity is produced 48 h after the administration of triolein (21). In Fig. 1 A are represented the individual values of \( K \) established, 48 h after triolein injection, in the 25 males and 25 females constituting the foundation stock from which the selective breeding was initiated. In this case, a sex effect is evident: the mean response to stimulation in females is about two times higher than in males. For each sex a considerable individual variation is observed.

These phenotypic variations reflect different genotypes since the selective breeding for triolein responsiveness has proved successful. The value of the phagocytic index \( K \), 48 h after triolein injection, is the phenotypic character chosen to carry out the selective breeding. Due to a scale effect, the frequency distribution curve of \( K \) value is skewed. However, a symmetrical frequency distribution can be obtained when \( K \) is expressed in natural logarithms (Fig. 1 B). Therefore, the genetic analysis will be made using \( \log n \) of \((K \times 1000)\).

In Fig. 2 are represented the mean values of \( K \), established in KTH and KTL mice during the selective breeding carried out for 26 consecutive generations. Considering the sex effect, males and females data have been represented separately. In both sexes, the genetic selection was successful, as shown by the progressive separation of high and low responder lines (Fig. 2). Considerable fluctuations of the \( K \) levels in successive generations are evident. These fluctuations are due to environmental factors that will be discussed later on. It should presently be noticed that these fluctuations are roughly parallel in the two
FIG. 1. Variations of responsiveness to stimulatory effect of triolein in the foundation stock. (A) Individual K values of the 25 ♂ and 25 ♀ constituting the foundation population. (B) Frequency distribution of log n (K x 1,000) values of the same animals.

FIG. 2. Progressive separation of KTH and KTL lines during 26 generations of selective breeding. The dotted lines indicate the mean values of unstimulated mice. Vertical bars indicate standard deviation.
lines and thus they do not interfere with the calculation of interline divergence produced by selective breeding.

The values of standard deviations indicated in Fig. 2 show that the interline difference was noticeable after five generations. At the 10th generation, the two lines are completely separated without overlapping. The interline separation increases progressively afterwards.

The genetic selection has increased the responsiveness of macrophages to triolein in F_{26} KTH mice of both sexes as compared with the foundation population. A still greater effect was produced in the decrease of responsiveness in KTL line. In fact, from F_{26} onwards, KTL mice are completely refractory to the stimulatory effect of triolein. In these generations the K values are often lower than those established in conventional untreated mice (dotted line). The physiological significance of this finding will be discussed later (see Tables II and III). The importance of the modifications produced by genetic selection in the phagocytic activity of macrophages may be better appreciated in Fig. 6 and Table III where the K values are indicated without transformation into n log values.

In Table I is reported a statistical analysis demonstrating the efficiency of the genetic selection in separating KTH and KTL lines. In this table, the total range of interline divergence is expressed in three ways: as a percentage of the initial population mean, in terms of the phenotypic standard deviation in the foundation population and in terms of the standard deviation of breeding value (i.e. the square root of the additive variance, VA, calculated as mentioned in Material and Methods).

The divergence between KTH and KTL mice of the 26th generation is about five times the standard deviation of the initial population. This difference is about 14 times the square root of the additive variance. A comparable efficiency of selective breeding has been reported by Falconer in his model of genetic selection for the character body weight at 6 wk in the mouse. In this case, the

| Sex   | Population | Log n (K × 1,000) | Interline divergence total range/foundation population* |
|-------|------------|-------------------|-------------------------------------------------------|
|       |            |                   | x | δP | δA |
| Male  | KTH F\textsubscript{26} | 4.66 ± 0.51       | P < 0.05 | 0.77 | 5.7 | 16.2 |
|       | Foundation | 3.78 ± 0.51       | P < 0.001 | 1.75 | 34.0 |
|       | KTL F\textsubscript{26} | 1.75 ± 0.34       | | | | |
| Female| KTH F\textsubscript{26} | 4.87 ± 0.44       | P < 0.01 | 0.61 | 4.1 | 12 |
|       | Foundation | 4.35 ± 0.64       | P < 0.001 | 2.24 | 25.0 |
|       | KTL F\textsubscript{26} | 2.24 ± 0.25       | | | | |

* Divergence between KTH and KTL F\textsubscript{26} divided by: x, the mean of the foundation population; δP, the phenotypic standard deviation of the foundation population; δA, the square root of the additive variance (VA) of the foundation population (VA = VP × h\textsubscript{A}).
response to selection was asymmetrical, namely, more pronounced downwards, as here-reported (Fig. 2, Table I).

The continuous variations of K in the foundation population (Fig. 1 A) and the progressive separation of KTH and KTL lines during the selective breeding (Fig. 2) clearly indicate that the character investigated is submitted to polygenic regulation.

**Genetic Analysis of KTH and KTL Mice.**

Estimation of realized heritability of the character. According to Falconer, the $h_2$ measures the percentage of superiority of the parents selected at each generation, that is transmitted to their progeny. The difference between the parents chosen and the mean of their generation is expressed by the selection differential $S$, which is a measure of the genetic pressure exerted by the selection. The response ($R$) to this genetic pressure is the difference between the offspring of the selected parents and the whole of the parental generation. Owing to the mentioned sex difference, the calculation was done, at each generation, by converting female values into male equivalents, i.e., by subtracting the sex difference found in the generation. The value of $h_2$ has been calculated for the KTH and KTL lines separately as well as from the interline divergence. In Fig. 3 are represented the cumulated values of $R$ and $S$ for the interline divergence throughout the selective breeding. The heritability of the character is definite but it is not very high ($h_2 = 12\% \pm 1$). Therefore, the 88% of the phenotypic variance are due to both non additive genetic and environmental variances. The rise of $R$ seems to be rather constant throughout the selection. From this, it would appear that the maximal interline separation was not yet obtained after 26 generations of selective breeding. Consequently, $F_{26}$ populations cannot be considered as

![Fig. 3. Calculation of heritability ($h_2$). Cumulated values of selection differential ($S$) and of response to selection ($R$) calculated from interline divergence throughout the selective breeding.](image)
completely homozygous for the character investigated and the selective breeding will be continued.

In Fig. 4 A, the interline divergence is represented by plotting the response to selection R against the cumulative selection differential S. In the same way, in Fig. 4 B are shown the slopes representing the h₂ calculated separately for each line. From the data of Fig. 4 B, it is obvious that the response to selection is asymmetrical. The decrease of responsiveness in KTL line occurs slightly faster than the increase of responsiveness in the KTH line. This is reflected by the value of h₂ which is respectively of 15% ± 3 and 8% ± 3.

Estimation of the number of independently segregating loci determining the character: responsiveness of macrophage to triolein. According to Falconer, an approximate evaluation of the number of loci, determining a given polygenic trait, may be proposed from the formula previously mentioned in Material and Methods (25).

The number of loci, resulting from this calculation, depends on the value of h₂ considered; it is of 27 when h₂ is calculated from the interline divergence (Fig. 4 A) and of 39 and 22 when h₂ is calculated respectively in KTH and KTL lines separately (Fig. 4 B).

On the basis of these data, it may be advanced that the character “responsiveness of RE macrophages to stimulatory effect of triolein” is a polygenic trait determined by the cumulative action of a group of about 27 independent loci (extreme values: 22-39 loci).

Distribution of macrophage responsiveness to triolein in interline crosses. The values of the phagocytic index K, established 48 h after triolein

![Fig. 4](image_url)
injection in \((KTH \times KTL)F_1\), in \(F_2\) and in backcross \((KTH \times F_1)\) and \((KTL \times F_1)\), are represented in Fig. 5. In all experiments an equivalent number of reciprocal crosses were made; the results being not significantly different, all the data were cumulated. These interline crosses have been obtained from the \(F_{28}\) generation of selective breeding. As it may be observed in Fig. 2, this generation gives a rather bad response in both lines, particularly in KTL as compared with the preceding generation \(F_{25}\). Since these are im predictable fluctuations of responsiveness in subsequent generations, the values of \(K\), established in both \(F_{25}\) and \(F_{26}\) are represented in Fig. 5. The mean values \(F_{25}-F_{26}\) were considered as the best estimation of \(KTH\) and \(KTL\) values (see Fig. 2). The data of Fig. 5 indicate that in both sexes the responsiveness of \(F_1\) hybrids is close to that of \(KTL\) mice. \(F_1\) hybrids show the usual sex effect. The \(K\) value of \((F_1 \times KTL)\) backcross is also similar to that of the low responder line while \((F_1 \times KTH)\) backcross are intermediate between high and low responders. The comparison of the distribution of interline crosses with the expected values in absence of dominance (indicated in Fig. 5) suggests a global dominance effect of low responsiveness in interline crosses. The degree of this dominance effect may be evaluated by comparing the mean of values obtained in \(F_1\) mice with both parental lines according to the formula presented in Material and Methods. The maximal theoretical value of \(VD/VA\), in absence of overdominance, is 0.5. From our data, this ratio is 0.34 in males and 0.38 in females. The dominance effect is therefore incomplete, as also suggested by the results obtained in \(F_1\) and backcrosses.

**Study of the Macrophage Stimulation Produced by Triolein in KTH and KTL Lines.** The above-stated results demonstrate that macrophage responsiveness to triolein is determined by a group of about 27 loci which can be separated by selective breeding. To study whether these loci are also involved in the regulation of the basic phagocytic function of RE macrophages in non-triolein-stimulated

![Graph showing distribution of log n (K x 1000) values in interline crosses. Approximately 20-40 mice in each group. Starred points indicate the theoretically expected values in absence of dominance.](image-url)
mice, the value of K was measured in KTH and KTL untreated mice of the 9th and 27th generations of selective breeding. The results obtained are represented in Table II where the K value of random bred albinos mice is also reported for comparison.

In KTH males, the basic phagocytic activity of macrophages was not significantly affected by selective breeding. However, in KTH females of the 27th generation, a high value of K is observed. The effect of the selective breeding on the basic phagocytic function of RE macrophages appears more clearly in the KTL mice of the 27th generation, which show a low value of K in both males and females. This effect might result from modifications in responsiveness of KTH and KTL macrophages to stimulatory lipids contained in food. A more detailed study of the phagocytic activity of RE macrophages in F27 KTH and KTL mice, 48 h after triolein injection, is reported in Table III.

The responsiveness of the selected mice to triolein is compared with that of conventional random bred albinos mice. The phagocytic activity is expressed by the index K which measures the total activity of macrophages bordering the blood stream, and by the corrected phagocytic index α which expresses this

### Table II

**Basic Phagocytic Activity of RE Macrophages in Unstimulated Mice**

| Mice    | Phagocytic index K |  |  |
|---------|-------------------|---|---|
|         | K                 | α | α |
| KTH     |                   |   |   |
| F9      | 0.017 ± 0.005 (12) | 0.024 ± 0.005 (8) |
| F27     | 0.021 ± 0.010 (5)  | 0.037 ± 0.005 (7) |
| KTL     |                   |   |   |
| F9      | 0.014 ± 0.004 (10) | 0.017 ± 0.004 (7) |
| F27     | 0.009 ± 0.001 (5)  | 0.011 ± 0.002 (5) |
| Random bred | 0.018 ± 0.007 (10) | 0.020 ± 0.008 (10) |

The figures in brackets indicate the number of mice in each group.

### Table III

**Phagocytic Activity of Macrophages, Measured 48 h after Triolein Administration, in Random Bred and F27 KTH and KTL Mice**

| Mouse strain | Sex | Nb of mice | κ | W/Wls | α | Liver mg/20 g | Spleen mg/20 g |
|--------------|-----|------------|---|-------|---|---------------|---------------|
| Random bred  | 5   | 10         | 0.075 | 16.8 | 7.1 | 1,100 ± 70 | 90 ± 30 |
|              | 6   | 10         | 0.090 | 17   | 7.6 | 1,080 ± 80 | 100 ± 40 |
| KTH F27      | 5   | 21         | 0.165 | 19.5 | 9.2 | 940 ± 95 | 87 ± 19 |
|              | 6   | 19         | 0.130 | 18.4 | 9.3 | 967 ± 104 | 118 ± 21 |
| KTL F27      | 5   | 18         | 0.006 | 22.4 | 4.1 | 840 ± 66 | 51 ± 19 |
|              | 6   | 20         | 0.010 | 20.7 | 4.4 | 880 ± 50 | 86 ± 35 |
activity per unit of weight of liver and spleen. The macrophages contained in these organs are responsible for about 95% of the total activity measured (13).

Responsiveness of macrophages to triolein has been substantially modified by selective breeding. Both $\kappa$ and $\alpha$ values are higher in KTH $F_{2}\alpha$ than in random bred albinos mice. The selection for low responsiveness is still more effective. In fact, KTL $F_{2}\alpha$ mice are completely unresponsive to triolein.

The values of index $\alpha$ indicate that the difference in responsiveness to triolein in KTH and KTL mice is mainly due to intrinsic modifications of the phagocytic activity of liver and spleen macrophages without important changes in the weight of these organs. Nevertheless, a significant loss in organ weight is observed in KTL mice.

Previous studies on macrophage stimulation produced by triolein in random bred albinos mice showed that the peak of the response occurred 48 h after a single injection and that repeated injections produced a higher macrophage stimulation (22). The kinetics of macrophage stimulation, produced by a single injection of triolein in KTH $F_{1}\alpha$ and KTL $F_{1}\alpha$, is represented in Fig. 6 A. In spite of the stronger effect in KTH mice, the dynamics of the response was not modified, the peak is reached at 48 h. In contrast, the very low response of KTL mice tends to increase until the 4th day. Three injections of triolein induced a stronger response in both $F_{2}\alpha$, KTH and KTL mice but the interline difference persists (Fig. 6 B). These results demonstrate that the genetic constitution of KTH and KTL mice regulates the responsiveness to triolein, independently of the dose administered.

**Discussion**

The phagocytic activity of macrophages is remarkably constant in physiological conditions. Nevertheless, stimulation or depression of this activity can be easily induced experimentally (20). These modifications reflect the high functional plasticity of these cells that have to cope with various environmental challenges.

The most potent stimulants of RE macrophages are certain bacteria or bacterial products (4, 10, 28) and lipid emulsions such as triolein. It must be pointed out that there exists a sharp difference between these two classes of stimulants. The effect of bacteria is a complex one, which involves, besides
macrophages, other cell types, namely, lymphocytes. Important modifications of the weight and histological structure of liver and spleen are produced (19, 29). At the level of macrophages, the effect of bacteria is also complex. Both phagocytic function and intracellular enzymes, responsible for the bactericidal effect, are modified. On the contrary, macrophage stimulation, induced by triolein, seems to be limited to both number and phagocytic function of macrophages (22) without important histological changes in the liver and spleen (29).

The action of lipid emulsions on the phagocytic activity of RE macrophages is strictly connected with the chemical structure of the lipids administered. Some lipids strongly stimulate the macrophages while others provoke a deep depression of their phagocytic activity. It was initially demonstrated that glyceryl trioleate is a potent stimulator while glyceryl mono-oleate is inactive. On the other hand, both ethyl stearate and ethyl oleate are depressors of macrophage phagocytosis, the effect of the former being much stronger than the later's one (21, 22).

Consecutive studies demonstrated that the maximal stimulation of macrophages was produced by insaturated triglycerides with a fatty chain length of 10 carbons (Tricaprin). When the fatty acid length is increased or decreased, a reduction in stimulatory activity occurs (30). Among the lipids, which drastically depress the macrophage phagocytosis, we find: ethyl stearate, cholesterol oleate, ethyl and methyl palmitate, and ethyl oleate (21, 31-33).

It is relevant to notice that the different fatty acids are normal constituents of the diet and that they may enter the portal circulation under the form of chylomicra resembling the lipid emulsions used in the above-mentioned studies. It was previously shown that chylomicra, with a high cholesterol content, are efficiently phagocytized by RE macrophages (11). The role played by the various food lipids in the regulation of the macrophages physiological activity should be specified but it would seem they are likely to intervene. The modifications in the basic phagocytic activity of macrophages produced by selective breeding (Table II) are in favor of this hypothesis. Experiments are in progress to investigate whether the same genes, controlling responsiveness to triolein in KTH and KTL mice, are also involved in the regulation of the depressory effect produced by the alkyl esters of fatty acids. The genetic regulation of macrophage responsiveness to triolein might suggest that an inherited factor intervenes in the familiar manifestations of altered metabolism of lipids and cholesterol (34) associated with certain types of cardiovascular diseases.

The genetic factors controlling the activity of macrophages in relation with immune responsiveness have recently been demonstrated. Two lines of mice have been separated by selective breeding for high and low antibody synthesis. In these mice, only selected for the character “antibody production”, the metabolism and processing of the antigen in the spleen macrophages have been drastically modified. The antigen persists in immunogenic form much longer in the macrophages of the high than in those of the low antibody producers (3, 35). The relationships of the genes determining the responsiveness of macrophages to triolein and those regulating the antigen metabolism in high and low immunoresponder mice are unknown. Studies on this problem are in progress.

The findings on genetic selection for responsiveness of RE macrophages to
triolein, here-reported, deserve some comments. The control of this trait is polygenic as demonstrated by the continuous variations of the K values in the foundation population and by the progressive interline separation observed during 26 generations of selective breeding. The sex effect, resulting in the higher responsiveness to triolein in the females, may be related to the previously demonstrated stimulatory effect of estrogens on the phagocytic activity of RE macrophages (23, 24). In fact, there is no significant sex difference in the basic macrophage activity in physiological conditions but macrophage responsiveness to various stimulants is often stronger in females than in males. In previous experiments, it was shown that the administration of living M. tuberculosis (BCG strain) produced an increase of K value higher in females than in males; estrogen-treated males, however, responded as well as females (36).

The reason for the parallel fluctuations of responsiveness to triolein, observed in both KTH and KTL mice during the selective breeding, is not clear (Fig. 2). It might come from seasonal variations in the macrophage activity or from different stimulatory efficiencies of lipid emulsions preparations due to small differences in the dispersion of lipid droplets in the medium.

From Fig. 4 B and Table I, it is evident that the effect of selective breeding is asymmetrical. It is more efficient in decreasing than increasing responsiveness. This asymmetry is reflected in the value of heritability which is of 8% ± 3 in KTH and 15% ± 3 in KTL; this difference is significant. The different factors accounting for asymmetrical response to selection are discussed in details by Falconer (25).

The findings reported in Fig. 5 suggest an incomplete dominance effect of low responsiveness. Usually, in the case of polygenic inheritance the F₁ hybrids show a mid-parental distribution. However, many examples of dominance or overdominance have been reported. For instance, in the polygenic regulation of mouse resistance to Salmonella typhimurium infection, the resistance is dominant in F₁ hybrids between a resistant and a susceptible strain (37). In lines of mice selected for high and low leukocyte counts, the genes associated with low leukocyte counts are dominant (38). In the genetic regulation of functional traits, the intensity of the stimulation may be determinant in the manifestation of dominance. High and low agglutinin production in mice immunized with sheep erythrocytes is a polygenic character determined by the interaction of a group of about 10 independent loci. In F₁ hybrids immunized with an optimal dose of antigen, high responsiveness is dominant, while low responsiveness is dominant when the hybrids are immunized with a threshold dose of antigen (39). In the present study, the distribution of F₁ in relation to the parental lines may also result from epistatic interaction between loci present at low frequency in the foundation population.

The heritability of the character investigated is relatively low ($h_s = 12\% ± 1$) but is well defined, since it is established from the data of 26 generations. The accuracy of the $h_s$ estimation is demonstrated by the small value of the standard deviations. Comparable values of $h_s$ have been reported in the literature for different polygenic characters such as: litter size in the mouse ($h_s = 15\%$) (27), age at puberty in female rats ($h_s = 15\%$) (40), viability in poultry ($h_s = 10\%$) (41).
The low value of heritability indicates that environmental factors account for a large proportion of the phenotypic variation. In spite of this, the interline separation was achieved, as demonstrated by the statistical analysis reported in Table I where the total range of interline separation was compared with the characteristics of the foundation population in the way proposed by Falconer (25). The success of the selective breeding for a character of such a low heritability means that the index K is an extremely precise measure of the phagocytic activity of RE macrophages. The character “responsiveness to triolein” is determined by the cumulative effect of a relatively large number (22–39) of independent loci. This explains why the variances of interline crosses (Fig. 5) differ from the theoretically expected ones, namely the abnormal low value of variance of F₂. A larger number of animals should be tested to find out the extreme phenotypes. Thus, the calculation of heritability from variance analysis in interline crosses would not be valid if done from the data presently available.

It should be reminded that the calculation of the number of independent loci, controlling the character investigated, is approximate and submitted to all the limitations of the method used. In the Falconer’s experiment of genetic selection for “6 wk body weight in the mouse”, the number of loci involved was estimated at about 35. It is of some interest to remark that our model of genetic selection for macrophage responsiveness to triolein is similar to the Falconer’s model of selection for “6 wk body weight in the mouse”. The efficiency of interline separation, the realized heritability and the number of independent loci involved are of the same order of magnitude in both cases.

Summary

The phagocytic index K, established from the rate of blood clearance of colloidal carbon, measures the phagocytic activity of RE macrophages in contact with the circulating blood.

The intravenous injection of glyceryl trioleate (triolein) produces a marked stimulation of the phagocytic activity of RE macrophages. This response is higher in the female than in the male mice.

The phenotypic character “responsiveness of macrophage to triolein” presents large individual variations in population of random bred albinos mice. This character is submitted to polygenic regulation.

Starting from a foundation population of 25 males and 25 females random bred albinos mice, two lines were separated by selective breeding for the character “responsiveness to triolein”: a “high” responder line, KTH, and a “low” responder line, KTL. After 26 consecutive generations of selective breeding, KTH mice present a very high response to triolein while KTL mice are almost irresponsive.

The heritability of this character (h²) calculated from the interline divergence is of 12% ± 1. This value of h² indicates that the character investigated is determined by the cumulative effect of a group of about 27 independently segregating loci.
The distribution of the character in \((KTH \times KTL)F_1\) and their backcrosses with parental lines suggests that low responsiveness is dominant over high responsiveness. The genetic regulation of responsiveness to triolein is independent from the dose administered. These results are discussed in relation to the importance of genetic factors controlling macrophage functions involved in lipid metabolism and in the specific and nonspecific mechanisms of immunity.

Received for publication 13 May 1974.

References

1. Askonas, B. A. and L. Jaroskova. 1970. Antigen in macrophages and antibody induction. In Mononuclear Phagocytes. R. van Furth editor. Blackwell Scientific Publications, Ltd. Oxford. 611.
2. Unanue, E. R. 1972. The regulatory role of macrophages in antigenic stimulation. Adv. Immunol. 15:95.
3. Biozzi, G., C. Stiffel, D. Mouton, Y. Bouthillier, and C. Decreusefond. 1974. La régulation génétique de la synthèse des immunoglobulines au cours de la réponse immunologique. Ann. Immunol. 125C:107.
4. Biozzi, G., B. Benacerraf, and B. N. Halpern. 1955. The effect of Salmonella typhi and its endotoxin on the phagocytic activity of the RES in mice. Br. J. Exp. Pathol. 35:226.
5. Biozzi, G., C. Stiffel, B. N. Halpern, and D. Mouton. 1960. Recherches sur le mécanisme de l'immunité non spécifique produite par les mycobactéries. Rev. Fr. Etud. Clin. Biol. 5:876.
6. Old, L. J., B. Benacerraf, D. A. Clark, E. A. Carswell, and E. Stockert. 1961. The role of the reticuloendothelial system in the host reaction to neoplasia. Cancer Res. 21:1281.
7. Cohn, Z. A. 1968. The structure and function of monocytes and macrophages. Adv. Immunol. 9:164.
8. Nelson, D. S. 1969. Macrophages and Immunity. North-Holland Publishing Co., Amsterdam.
9. Allison, A. C. 1970. On the role of macrophages in some pathological processes. In Mononuclear Phagocytes. R. van Furth editor. Blackwell Scientific Publications, Ltd. Oxford. 422.
10 Stiffel, C., D. Mouton, and G. Biozzi. 1971. Rôle des macrophages dans l'immunité non spécifique. Ann. Inst. Pasteur. (Paris). 120:412.
11. Neveu, T., G. Biozzi, B. Benacerraf, C. Stiffel, and B. N. Halpern. 1956. Role of RES in blood clearance of cholesterol. Am. J. Physiol. 187:269.
12. Carr, I. 1973. The macrophage. A review of ultrastructure and function. Academic Press, Inc. New York. 99.
13. Biozzi, G., B. Benacerraf, and B. N. Halpern. 1953. Quantitative study of the granuloplectic activity of the RES. A study of the kinetics of the granuloplectic activity of the RES in relation to the dose of carbon injected. Relationship between the weight of the organs and their activity. Br. J. Exp. Pathol. 34:441.
14. Biozzi, G., B. Benacerraf, C. Stiffel, and B. N. Halpern. 1954. Etude quantitative de l'activité granulopexique du SRE chez la souris. C. R. Seances Soc. Biol. Fil. 148:431.
15. Biozzi, G., and C. Stiffel. 1965. The physiopathology of the reticuloendothelial cells of
the liver and spleen. In Progress in Liver Diseases. H. Popper and F. Schaffner editors., Grune and Stratton, Inc. New York. 166.

16. Stiffel, C., D. Mouton, Y. Bouthillier, C. Decreusefond, and G. Biozzi. 1970. Réponse du SRE au Mycobacterium tuberculosis (BCG) et au Corynebacterium parvum chez des souris de différentes lignées. J. Reticuloendothelial Soc. 7:280.

17. Buschman, H. 1973. Causes of the variation of phagocytic activity in mice and the role of genetic influences. In Non Specific Factors Influencing Host Resistance. W. Braun and J. Ungar editors. S. Karger, AG, Basel. 137.

18. Biozzi, G., B. Benacerraf, F. Grumbach, B. N. Halpern, J. Levaditi, and N. Rist. 1954. Etude sur l'activité granulopoïétique du SRE au cours de l'infection tuberculeuse chez la souris. Ann. Inst. Pasteur (Paris). 87:291.

19. Halpern, B. N., A. R. Prevot, G. Biozzi, C. Stiffel, D. Mouton, J. C. Morard, Y. Bouthillier, and C. Decreusefond. 1964. Stimulation de l'activité phagocytaire du SRE provoquée par Corynebacterium parvum. J. Reticuloendothelial Soc. 1:77.

20. Stiffel, C., G. Biozzi, and D. Mouton. 1970. Kinetics of the phagocytic function of reticuloendothelial macrophage in vivo. In Mononuclear Phagocytes. R. van Furth. editor. Blackwell Scientific Publications, Oxford. 335.

21. Stuart, A. E., G. Biozzi, C. Stiffel, B. N. Halpern, and D. Mouton. 1960. The stimulation and depression of reticuloendothelial phagocytic function by simple lipids. Br. J. Exp. Pathol. 41:599.

22. Biozzi, G., C. Stiffel, and D. Mouton. 1963. Stimulation et dépression de la fonction phagocytaire du système réticuloendothélial par des émulsions de lipides. Relations avec quelques phénomènes immunologiques. Rev. Fr. Étud. Clin. Biol. 8:341.

23. Biozzi, G., B. N. Halpern, D. L. J. Bilbey, C. Stiffel, B. Benacerraf, and D. Mouton. 1957. Oestrogènes et fonction phagocytaire du SRE. C. R. Seances Soc. Biol. Fil. 151:1326.

24. Nicol, T., and D. L. J. Bilbey. 1960. The effect of various steroids on the phagocytic activity of the RES. Reticuloendothelial Structure and Function. The Ronald Press Co., New York. 301.

25. Falconer, D. S. 1960. Introduction to quantitative genetics. The Ronald Press, New York. Chap. 11.

26. Cavalli-Sforza, L. L., and W. F. Bodmer. 1971. The genetics of human population. W. H. Freeman and Company, San Francisco. Chap. 9.

27. Falconer, D. S. 1955. Patterns of response in selection experiments with mice. Cold Spring Harbor Symp. Quant. Biol. 20:178.

28. Halpern, B. N., G. Biozzi, B. Benacerraf, and C. Stiffel. 1957. Physiopathology of the Reticuloendothelial System. Blackwell Scientific Publications, Ltd. Oxford. 204.

29. Stuart, A. E. 1970. The Reticuloendothelial System. E. & S. Livingstone, Ltd. Edinburgh. Chap. 3.

30. Cooper, B. N. 1964. Functional modification of reticuloendothelial cells by simple triglycerides. J. Reticuloendothelial Soc. 1:50.

31. Stuart, A. E., and A. E. Davidson. 1964. Effect of simple lipids on antibody formation after injection of foreign red cells. J. Pathol. Bacteriol. 87:305.

32. Di Luzio, N. R., and D. A. Blickens. 1966. Influence of intravenously administered lipids on reticuloendothelial function. J. Reticuloendothelial Soc. 3:250.

33. Neill, V. M., L. J. Cole, and R. M. Hyde. 1972. Comparison of the reticuloendothelial system of normal and lipid-treated BALB/C and C57BL/6 mice. J. Reticuloendothelial Soc. 12:436.

34. Stiffel, C., D. Mouton, Y. Bouthillier, C. Decreusefond, and G. Biozzi. 1966. Activité fonctionnelle du système réticuloendothélial chez des souris obèses. J. Reticuloendothelial Soc. 3:236.
35. Weiner E., and A. Bandieri. 1974. Differences in antigen handling by peritoneal macrophages from the Biozzi High and Low responder lines of mice. *Eur. J. Immunol.* 4: 457.

36. Halpern, B. N., C. Stiffel, G. Biozzi, and D. Mouton. 1960. Influence des hormones sexuelles sur la stimulation de la fonction phagocytaire du système réticuloendothélial provoquée par l’inoculation du bacille de Calmette-Guérin (BCG) chez la Souris. *C. R. Seances Soc. Biol. Fil.* 154: 1994.

37. Gowen, J. W. 1963. Genetics of infectious diseases. In *Methodology in Mammalian Genetics*. W. J. Burdette, Editor. Holden Day Ing. San Francisco. 383.

38. Chai, C. K., 1970. Genetic basis of leucocyte production in mice. *J. Hered.* 61:67.

39. Stiffel, C., D. Mouton, Y. Bouthillier, A. M. Heumann, C. Decreusefond, J. C. Mevel, and G. Biozzi. Polygenic regulation of general antibody synthesis in the mouse. Progress in Immunology, Vol. II. Proceedings of 2nd International Congress of Immunology. ASP Biological and Medical Press, Amsterdam. In press.

40. Warren, E. P., and R. Bogart. 1952. Effect of selection for age at time of puberty on reproductive performance in the rat. *Sta. Tech. Bull. Ore. Agric. Exp. Sta.* 25:27.

41. Robertson, A., and I. M. Lerner, 1949. The heritability of all or none traits viability of poultry. *Genetics.* 34:395.