THE ROLE OF MACROPHAGES IN THE GENERATION OF T-HELPER CELLS

II. The Genetic Control of the Macrophage-T-Cell Interaction for Helper Cell Induction with Soluble Antigens

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In a recent communication, we demonstrated the requirement for macrophages in the generation of T-helper cells with either soluble or particulate antigen (1, 2). The form of antigen influenced the manner of T-cell macrophage interaction—with particulate antigen, allogeneic macrophages or 2-Mercaptoethanol (2ME)1 were effective. In contrast, only syngeneic (or F1) macrophages were effective with soluble antigen, but 2ME, over a range of concentrations, was ineffective.

Several immune reactions (3), such as T-B collaboration (4) and the killing of virus-infected cells (5) have been shown to be linked to the H-2 region of the major histocompatibility locus. It was therefore of interest to determine whether the macrophage-T-cell interactions in helper cell induction using soluble antigens is also controlled by the H-2 complex, and if so, by which subregion of H-2. The results showed that the inability of allogeneic macrophages to participate in helper cell induction with soluble antigens was not due to the presence of suppressor cells generated in these cultures but was due to a genetic restriction of the macrophage-T-cell interaction which was governed by the I-A region of the H-2 complex.

Materials and Methods

Animals. CBA mice were either bred at University College or at the Imperial Cancer Research Fund animal unit at Mill Hill, London. BALB/c mice were obtained from the latter. Mouse strains congenic to C57BL/10, B10.D2, B10.A(4R), B10.A(5R), B10. BR, and DBA/1 mice were donated by Dr. Elizabeth Simpson, Clinical Research Center, Harrow. Some B10.A and B10.BR mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. AQR and A.TL mice were provided by Dr. Hilliard Festenstein, London Hospital. Some of the B10, B10.D2, B10.A, and B10.BR mice used were

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1 Abbreviations used in this paper: APC or PFC, antibody-forming cells; DNP-POL, DNP conjugated to polymeric flagellin from Salmonella adelaide; H-2 region, major mouse transplantation locus, K, I, SS-Sip, and D are the major subdivisions, with I further subdivided into I-A, I-B, I-C; KLH, keyhole limpet hemocyanin; Mph, macrophage; TGAL, poly-L(Tyr, Glu)poly-D, L-ala-poly-L-lys; TNP-KLH, TNP conjugated to KLH; TNPT, TNP conjugated to coliphage T.

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bred at University College. The H-2 region of all the mouse strains used is shown in Table I. Mice were used between 60 and 180 days of age.

Antigens. The antigens used were either keyhole limpet hemocyanin (KLH) donated by Dr. M. B. Rittenberg (University of Oregon Medical School) or poly-t(Tyr,Glu)-poly-d-Ala-poly-L-lys (TGAL) donated by Dr. E. Moses (The Weizmann Institute of Science, Rehovot, Israel). KLH in different amounts (1 mg, 5 mg, 10 mg) was conjugated to Sepharose 213 using cyanogen bromide (18). The methods used for di-or trinitrophenylation of KLH and TGAL were described previously (1). Conjugates used had 14 groups of TNP per 100,000 daltons of KLH, or four groups of DNP per 150,000 of TGAL. Dinitrophenylated flagellin (DNP-POL) was prepared as described previously (1). Trinitrophenylated phage T4 (TNP-T4) was a gift from Dr. M. B. Rittenberg.

TABLE I

H-2 Region of the Mouse Strains Used*

| Strain         | H-2 haplotype | H-2 region | I region | I-A‡ | I-B‡ | I-C | S | D |
|----------------|---------------|------------|----------|------|------|-----|---|---|
| CBA            | k             | k          | k        | k    | k    | k   | k | k |
| C57/BL.10      | b             | b          | b        | b    | b    | b   | b | b |
| BALB/c         | d             | d          | d        | d    | d    | d   | d | d |
| B10.D2         | d             | d          | d        | d    | d    | d   | d | d |
| B10.BR         | k             | k          | k        | k    | k    | k   | k | k |
| DBA/2          | q             | q          | q        | q    | q    | q   | q | q |
| B10.A          | a             | k          | k        | k    | k    | d   | d | d |
| B10.A(4R)      | h             | k          | k        | k    | b    | b   | b | b |
| B10.A(5R)      | i             | b          | b        | b    | d    | d   | d | d |
| AQR            | y             | q          | k        | k    | d    | d   | d | d |
| A.TL           | t             | s          | k        | k    | k    | k   | d | d |

Vertical bars show cross-over positions within the H-2 region.
*Taken from Shreffler and David (14).
‡Previously known as Ir-IA and Ir-IB.

Preparation and Use of Antisera. A sheep antimouse T-cell serum (anti-T) was prepared by Dr. I. McConnel (Royal Postgraduate Medical School, Hammersmith) and adsorbed and tested and used as described earlier (9).

Tissue Culture Conditions. The Marbrook-Diener flasks and media used were described in detail previously (2). The cultures were performed as described elsewhere (9) except with some modifications in the first stage of culture, the induction of helper cells. To induce helper cells (a) the T-cell source was depleted of adherent cells by one of the methods described above, (b) a known number of peritoneal exudate (PE) macrophages was added, and (c) T cells and macrophages were incubated together with the carrier protein for 4 days. As a control the adherent cell-depleted T cells alone were incubated with the carrier protein. Cultures were performed as described in the previous paper (2). The medium for cultures of lymphocytes from mice other than the CBA strain was enriched with 2-ME at a final concentration of $5 \times 10^{-4}$ M, because in its absence lymphocytes of the other mouse strains used (except CBA) respond poorly to soluble antigens. The magnitude of the usual cooperative anti-DNP-response using unprimed CBA spleen cells and in vitro-induced (or in vivo-induced) helper cells ranges from 200 to 500 PFC/culture. The average background, in the absence of helper cells, or of antigen was about 20-60 PFC. The response of lymphocytes from other mouse strains is lower, ranging from 150 to 250 PFC/culture, provided the cultures contain 2-ME. The average background of these cultures, however, is lower, about 10-30 PFC. All experiments shown in the Tables were performed three to five times except for the experiments shown in Table VII (Identity at the I-A locus) and Table VIII (Exclusion of the K-locus) which were performed twice.
**Preparation of Lymphoid Cells.** Spleen and lymph node cells were used and prepared as described elsewhere (9).

**Macrophages.** PE was harvested from normal mice or mice injected with 1 ml of proteose peptone (Oxoid, London) or 2 ml of a solution of 2% starch 3 or 4 days earlier. No functional differences were detected between macrophages obtained from stimulated or unstimulated mice. Stimulated PE contained between 75 and 85% phagocytic cells, whereas in unstimulated PE only about 50-60% of the cells were phagocytic. In some experiments PE cells were purified by treatment with either an anti-T serum and complement, or anti-T and anti-B serum and complement, or irradiation with 2,000 rad or with a combined treatment of irradiation (2,000 rad) and antisera, the latter treatment yielding 99.7% cells taking up latex beads. However, no functional difference was found between unpurified, partially purified, or highly purified PE cells in the test system used.

**Removal of Adherent Cells from Spleen or Lymph Node Cells.** Phagocytic spleen cells were allowed to take up iron particles and then removed by means of a strong magnet as described earlier (1). This method removed, besides phagocytic cells, a considerable number of B cells as tested by the ability of the iron magnet-treated spleen cells to mount an antibody response against thymus-independent antigens, which was reduced up to 90%, or by staining with rabbit antimouse Ig. The recovery of cells after the iron magnet treatment was about 40-50%. Since this method was equally effective as using nylon wool or polystyrene beads (2), the iron magnet method was mostly used, because of its rapidity and simplicity.

**Antibody-Forming Cell (AFC) Assays.** The AFC assays were performed as described elsewhere (1). Only direct, IgM were assayed, since no IgG AFC were found in cultures using unprimed B cells.

**Statistics.** The results are given as arithmetic means of the DNP-AFC per culture ± standard error (SE). Levels of significance were calculated by Student's t test, using a program devised by Dr. S. H. Leech, University College, and a Hewlett Packard 9810A Calculator (Packard Instrument Co., Downers Grove, Ill.).

**Results**

**Inability of Allogeneic Macrophages to Interact with T Cells.** 10^6 CBA or BALB/c macrophages, which had been treated with anti-T serum and complement, were cultured with KLH and purified T cells obtained from lymph nodes for 4 days, then various numbers of viable cells from each culture were added to T-depleted CBA spleen cells and cultured with TNP-KLH, and the anti-DNP response measured 4 days later. Table II shows that helper cells were generated only with syngeneic macrophages, but not with allogeneic macrophages even if the number of viable (helper) cells transferred ranged from 10^4 to 10^6.

**Inability of Allogeneic Macrophages to Effectively Interact with T Cells in Helper Cell Induction is not due to a Malfunction of the Macrophages.** The possibility exists that the inability of allogeneic macrophages to cooperate with CBA T cells in helper cell induction was due to the malfunction of these macrophages. This was ruled out by reciprocal experiments, in which the function of allogeneic macrophages was tested by incubation with their histocompatible T cells and KLH (Table III). B10.D2 macrophages were not able to facilitate helper cell induction if cultured with CBA T cells and KLH but were fully functional if incubated with B10.D2 T cells and KLH. Conversely, CBA macrophages were unable to generate helper cells when incubated with B10.D2 T cells and KLH, but did so when incubated with CBA T cells and KLH.

**Allogeneic Macrophages Incubated with T Cells and KLH do not Generate Suppressor Cells.** The incapacity of allogeneic macrophages for helper cell induction could also have been caused by suppressor cells generated by incubation of T cells with allogeneic macrophages and KLH. Experiments shown in Table III exclude this possibility. CBA and B10.D2 macrophages were
incubated together with either CBA or B10.D2 T cells and KLH, then $3 \times 10^6$ viable cells were added to either CBA or B10.D2 spleen cells and TNP-KLH. The anti-DNP response measured 4 days later was normal, indicating that there was no suppression of helper activity, even when helper cells were generated in the presence of twofold more allogeneic than syngeneic macrophages. The PFC response was also not abolished when CBA T cells were incubated separately with either CBA or B10.D2 macrophages and KLH, and tested for suppressor cells in the following manner: $3 \times 10^6$ viable cells from each culture were added together to CBA spleen cells and TNP-KLH. The results in Table III indicate that the helper activity generated by the syngeneic macrophages did not mask putative suppressor activity generated in the presence of allogeneic macrophages.

Demonstration of H-2-Linked Restriction of the Macrophage T-Cell Interaction in Helper Cell Induction. The lack of helper cell induction with allogeneic macrophages showed that the macrophage T-cell interaction is genetically restricted. It was therefore of interest to test if this restriction is H-2-linked, as was demonstrated for other cell interaction (4). Thus, macrophages from B10.BR, B10.D2, B10.A, and B10 mice were cultured with KLH and CBA T cells, 4 days later $3 \times 10^6$ viable cells from each culture were added to CBA spleen cell cultures containing TNP-KLH. After 4 days the anti-DNP response was measured. In the presence of B10.BR or B10.A macrophage helper cells were generated, but not with B10.D2 or B10 macrophages (Table IV). All these strains are congenic to C57BL/10 mice (14). B10.BR and CBA have the same H-2 complex on a different background, whereas B10.D2 and B10 have different H-2
complexes on the same background. The results thus show that the genetic restriction of the macrophage T-cell interaction is H-2-linked. Moreover, the induction of T-helper cells in the presence of B10.A macrophages demonstrates that only the K portion of the H-2 complex is involved, as defined by the recombinant chromosome H-2a. To examine any undetected involvement of the D-end of the H-2 region, B10.A macrophages were incubated together with B10.D2 T cells and KLH, a combination which only shares this part of the H-2 region. There was no helper cell induction (Table V).

**Mapping of the Genetic Restriction of the Macrophage T-Cell Interaction in Helper Cell Induction.** The K-end of the H-2 complex is known to contain the K-, I-A, and I-B regions and thus the restriction must be determined by one of these three regions. To map the restriction, other mouse strains containing H-2 recombinant chromosomes were tested in the same way as above. Additionally, another soluble antigen, TGAL, was also tested to ensure that this genetic restriction was not only found with KLH. Table VI shows that B10.A(4R), ATL, and AQR macrophages were also effective when incubated with CBA T cells in generating helper cells to KLH or TGAL. B10.A(4R) macrophages share the K- and the I-A region of the H-2 complex of the CBA T cells, whereas A.TL and AQR macrophages share the I-A region, but differ at the K-region and other regions not in the K-end of the H-2 complex. This suggests the possibility that
identity only at the I-A region is necessary for a successful macrophage T-cell interaction in helper cell induction with soluble antigens. But this assumption is only valid if only a single genetic locus is involved. It is possible that there is gene duplication and that the restriction lies in two or even three regions of the H-2 complex, but identity at a single region is sufficient for successful interaction. To exclude the other possibilities strain combinations were chosen which only share either the K-, the I-A or the I-B region and differ at all other regions of the H-2 complex. Tables VII and VIII show the results. The generation of helper cells by incubation of B10.A(4R) T cells and AQR macrophages and KLH, a combination which only shares the I-A region shows that identity at this single locus is sufficient for a successful macrophage T-cell interaction (Table VII). On the other hand, sharing of only the I-B locus does not allow a successful macrophage-T-cell interaction as shown by the negative result of the combination of B10.A(4R) T cells and B10.A(5R) or B10 macrophage (Table VI) or the reciprocals, B10 T cells and B10.A(4R) macrophage (Table VIII), the latter combination sharing in addition to I-B also the D-end of the H-2 region. A similar negative result was obtained by using a combination which shares only the K-region DBA/1 T cells and AQR macrophages (Table VIII). Therefore, the macrophage T-cell restriction for helper cell induction with KLH and TGAL is genetically controlled by the I-A region.

Discussion

The results obtained previously (1, 2) and extended in this communication indicate that helper cell induction to nonparticulate antigens does not occur in the absence of a certain genetic similarity between collaborating T cells and
### Table V

**Genetic Restriction of the Macrophage-T Cell Interaction in Helper Cell Induction Lies in the Left Hand Part (K, 1-A or 1-B) of the H-2 Region**

| T cells (Mph depleted spleen) | Ag | Mph (5 x 10^6) | H-2 region of Mph | Helper cells added | Source of spleen | Challenge (AFC ± SE) |
|------------------------------|----|----------------|-------------------|-------------------|------------------|---------------------|
| CBA | KLH 0.1 | NIL | | " | " | CBA TNP-KLH 0.1 | 23 ± 23 |
| " | " | CBA | kkkkkk | " | " | " | 615 ± 198 |
| " | " | B10.A | kkkddd | " | " | " | 457 ± 99 |
| " | " | B10.A2 | dddddd | " | " | " | 50 ± 15 |
| B10.D2 | " | NIL | | " | " | CBA TNP-KLH 0.1 | 30 ± 25 |
| " | " | B10.D2 | dddddd | " | " | " | 280 ± 5 |
| " | " | CBA | kkkkkk | " | " | " | 43 ± 12 |
| " | " | B10.A | kkkddd | " | " | " | 53 ± 23 |

*Results taken from the same experiment as in Table III.

\(\dagger\) As in Table IV.

\(\ddagger\) 2 x 10^6 particles/ml.

### Table VI

**Mapping of the Genetic Region Controlling the Macrophage-T Cell Interaction in Helper Cell Induction with Soluble Antigen**

| T cells (Mph depleted spleen) | Ag | Mph (5 x 10^6) | H-2 region of Mph | Helper cells added | Source of spleen | Challenge (AFC ± SE) |
|------------------------------|----|----------------|-------------------|-------------------|------------------|---------------------|
| + | KLH 0.1 | NIL | | " | " | CBA TNP-KLH 0.1 | 73 ± 37 |
| + | " | CBA | kkkkkk | " | " | " | 306 ± 62 |
| + | " | B10.A | kkkddd | " | " | " | 293 ± 52 |
| + | " | B10.A(4R) | kbbbb | " | " | " | 283 ± 33 |
| + | " | A.TL | skkkkd | " | " | " | 327 ± 73 |
| + | " | AQR | qkkddd | " | " | " | 303 ± 43 |
| + | " | B10.D2 | dddddd | " | " | " | 63 ± 29 |
| + | " | B10 | bbbbb | " | " | " | 70 ± 17 |
| + | TGAL 1.0 | NIL | | + | " | DNP-TGAL 1.0 | 63 ± 14 |
| + | " | CBA | kkkkkk | + | " | " | 217 ± 13 |
| + | " | B10.A | kkkddd | + | " | " | 283 ± 69 |
| + | " | B10.A(4R) | kbbbb | + | " | " | 217 ± 26 |
| + | " | AQR | qkkddd | + | " | " | 237 ± 69 |
| + | " | B10 | bbbbb | + | " | " | 57 ± 27 |
| | | | | | " | " | 27 ± 36 |
| | | | | | " | " | 88 ± 31 |
| | | | | | " | " | 395 ± 73 |
| | | | | | " | " | 70 ± 26 |
TABLE VII
Identity at the I-A locus is Necessary for a Successful Macrophage-T Cell Interaction in Helper Cell Induction with Soluble Antigen

| T cells (Mph depleted spleen) | Ag    | Mph | H-2 region | Source of spleen | Challenge | (AFC ± SE) |
|------------------------------|-------|-----|------------|------------------|-----------|------------|
| B10.A(4R) KLH 0.1           | NIL   |     |            | B10.A(4R)       | 3 x 10^9  | 12 ± 3     |
| "                            | "     | B10 | bbbb      | "                | "         | 217 ± 22   |
| "                            | "     | AQR | qkkd      | "                | "         | 200 ± 48   |
| "                            | "     | B10 | bbbbbb    | "                | "         | 3 ± 3      |
| "                            | "     | "   | "         | "                | "         | 10 ± 6     |

*2 x 10^8 particles/ml.

TABLE VIII
Identity Solely at the K- or I-B Locus is not Enough for Macrophage-T Cell Interaction in Helper Cell Induction with Soluble Antigen

| T cells (Mph depleted spleen) | Ag    | Mph | H-2 region | Source of spleen | Challenge | (AFC ± SE) |
|------------------------------|-------|-----|------------|------------------|-----------|------------|
| B10 KLH 0.1                  | NIL   |     |            | B10              | 10^9      | 27 ± 7     |
| "                            | "     | B10 | bbbb      | "                | "         | 177 ± 55   |
| "                            | "     | "   | "         | "                | "         | 0          |
| "                            | "     | DBA/1| qqqqqq | DBA/1            | "         | 140 ± 30   |
| "                            | "     | AQR | qkkd      | "                | "         | 20 ± 15    |
| "                            | "     | B10 | bbbbbb    | "                | "         | 20 ± 11    |
| "                            | "     | "   | "         | "                | "         | 7 ± 7      |
| "                            | "     | "   | "         | "                | "         | 7 ± 3      |
| "                            | "     | "   | "         | "                | "         | 7 ± 7      |

*2 x 10^8 particles/ml.

This need for a genetic similarity was absolute and independent of the number of allogeneic macrophages. It was not due to a functional defect in these macrophages as they were effective with T cells from their appropriate mouse strains. These results are essentially similar to those obtained by Rosenthal and Shevach, measuring antigen-induced lymphocyte proliferation in the guinea pig (6, 7). In the present study the genetic restriction was shown with two different soluble antigens, one, KLH, whose response is not under obvious genetic control, and the other, TGAL, under H-2-linked genetic control (8); other
antigens are being tested. It was also shown in several strain combinations, involving mice with different H-2 genotypes (CBA, B10.D2, B10, B10.A(4R), ASW, DBA/1). Thus, the results may be generally true for other antigens and other mouse strains. There are several possible explanations for this requirement of genetic similarity in helper cell induction.

First, it could be argued that genetically dissimilar macrophages do not function because they are “rejected” and are rendered nonfunctional by the allogeneic T cells. This possibility is excluded since allogeneic macrophages survive in culture and do function (i.e. are not rejected) provided a particulate antigen is used, such as KLH conjugated to Sepharose. Furthermore, since macrophage supernates are also effective (1, 2) it is difficult to envisage how T cells would render the molecules in the supernate nonfunctional. An even more rigorous exclusion of the concept of rejection was obtained by using stable radiation chimeras which are cross-tolerant (Sprent and von Bohmer, J. Exp. Med. 1975. 141:322). In this instance also, allogeneic macrophages were not effective (unpublished observations).

Secondly, it is possible that the lack of response is due to the generation of suppressor cells instead of helper cells in cultures containing allogeneic macrophages. This concept was tested and excluded (Table III). Two experimental systems were used: culturing T cells with both syngeneic and allogeneic macrophages, and mixing T cells which had been cultured with allogeneic macrophages with T cells cultured with syngeneic macrophages. In both instances, even if the allogeneic macrophages or T cells cultured with allogeneic macrophages were present in excess, there was no suppression of the response of the helper cells. Since we know that suppressor cells may be induced and expressed in our culture conditions (Kontiainen and Feldmann, unpublished data) these tests exclude the possibility that suppressor cells account for the requirement for genetic similarity.

A third possibility is that T cells and macrophages must share a certain genetic region. This hypothesis is favored by the data reported above. It was of interest that F1 macrophages were not as effective as the parental strains (1), suggesting the existence of a gene dosage effect. This has also been noted in the studies of antigen-induced proliferation (7).

In contrast to our studies and those of Rosenthal and Shevach (6, 7) some workers have published data which suggests that allogeneic macrophages can function in the generation of antibody (10, 11). The reasons for this difference are not clear as helper cells must be induced to permit thymus-dependent antibody responses. In some experiments it is possible that the residual syngeneic macrophages were functional (10), in other experiments it is possible that help was provided by an allogeneic effect, in response to allogeneic macrophages (11).

The genetic region involved in T-macrophage interaction was mapped using congenic mouse strains, varying only in the H-2 complex, and strains with intra H-2 recombinations. The results in Tables III and IV indicate that the gene involved is in the H-2 region. By the use of B10.A recombinant, macrophages (H-2b or H-2a/a) which cooperated with B10.BR or CBA (H-2b) T cells, but not with B10.D2 (H-2b) T cells, the genetic locus involved was mapped in the K or I regions of the H-2 complex.
Further definition of the genetic locus involved was made by testing a range of macrophages from mice with other recombinant H-2 chromosomes. Such an experiment is shown in Table VI. Since the B10.A(4R) shares only the K and I-A region of the H-2 complex with CBA, and AQR and A.TL share the I but not the K region with CBA, this experiment indicates that the relevant locus is in the I-A region. This conclusion is only valid, however, if it is assumed that there is only a single genetic locus in the H-2 complex which could mediate the relevant interaction. By analogy with lymphocyte stimulation determinants which map in at least two regions of the H-2 complex (12, 14) the above genetic mapping of the T-macrophage cell interaction may not be sufficient, and it was necessary to formally exclude the possibility that identity at the K or I-B region alone was sufficient. Similarity at the other regions I-C, SS-Slp, and D had been clearly excluded (with the antigens tested) in the experiments with B10.A macrophages and B10.D2 T cells (Table IV) and with B10.A(4R) T cells and B10 macrophages (Table VII) or the reciprocal cell combination (Table VIII).

Evidence confirming the mapping of the T-macrophage cooperation locus in the I-A region is shown in Table VII, using the B10.A(4R) T cell/AQR macrophage combination which only shares the I-A* region of the H-2 complex. (or using B10.A(4R)/A.TL, data not shown). However, the T-macrophage locus is probably also in the I-A region of mice of the H-2* genotype as exemplified by B109 (Table VII), or in the H-2* genotype of B10.D2* (Table V). Identity at the I-B locus only was excluded by experiments with B10/B10.A(4R) the B10.A(4R) B10.A(5R), and the B10.A(5R)/D2.GD combinations.4 Identity at the K locus itself was shown to be insufficient using two pairs of mice, the DBA/1 and AQR combination (K+) and the A.SW/A.TL (K+5) combination.4 Taken together, the evidence is strong that T-macrophage cooperation is determined by genes in the I-A region. It would be of interest to know if this was also true for antigens under the H-2-linked genetic control of the I-B region. Since Katz et al. (13) have mapped their T-B interaction locus in the I (I-A or I-B) region, it is possible that the two loci may be identical.

A pertinent question is the relationship of the T-macrophage interaction locus to the other functions which map in the I genetic region. This region controls in codes for: (a) H-2-linked immune response genes (3); (b) I-region-associated (Ia) antigens, which are predominantly expressed on B cells and macrophages (14-16); (c) lymphocyte-determined antigens, which stimulate strong mixed lymphocyte responses (12); (d) cell interaction between T cells and B cells (4, 13); and (e) a factor involved in cell cooperation, putatively released by T cells (17).

The size of the I region is not known (18), but it is sufficient to permit multiple functions to be expressed. Some of these functions, such as the immune response genes, have a degree of immunological specificity, and as such imply the presence of multiple genes. In a similar way there are > 15 known Ia specificities (D. Schreffler, personal communication) and probably more will be discovered.

Mapping in K or I-A.

Mapping in K, I-A, or I-B.

Data not shown.
Thus, it seems important to consider simplifying hypotheses which reduce the number of genes (or products) needed to account for the six functions mapping in that region (Ir genes, Ia antigens, LD antigens, cell interaction T/B and T/M, and "T factor").

The first speculation is that the factor reported by Munro et al. (17) may be identical to the genetically related factor we have described (15), (Erb and Feldmann, manuscript in preparation). The reasons for this are as follows: (a) the factor is obtained from activated T-cell spleen, which contains up to 20% macrophages. T-cell origin was only inferred by sensitivity to anti-θ serum, which does not exclude a T-cell-dependent macrophage factor; (b) the assay system used, lethally irradiated mice repopulated with bone marrow contains many T cells (radioresistant T cells, T cells in marrow, T-cell differentiating from marrow precursors). Thus, the factor reported could act in the development of helper cells, as reported above. This possibility is supported by the fact that this unstable factor (Taussig, personal communication) caused a response which peaked late, at days 12-14. Furthermore, both factors were elicited by the same antigen (TGAL) even in nonresponder mice (15, 17).

Genetic restrictions of T-B interaction have been reported and mapped also in the I region (I-A or I-B) (13). Thus it is possible that the same genes control both T-B and T-macrophage interaction, possibly through the same mediator.

The relationship of Ia antigens, Ir genes, and the macrophage factor involved in T-macrophage interaction is of interest. Preliminary absorption experiments suggest that the macrophage factor responsible for the genetic restrictions contains Ia determinants and part of the immunogen. It is conceivable that the function of H-2-linked immune response genes is mediated by these Ia antigen-immunogen complexes, with a particular Ia antigen, or combination of Ia antigens facilitating the response to a class of antigens. Clearly, more definitive concepts of the nature and function of the macrophage and other products, coded for by the I region, await further experimental analysis.

Summary

Helper cell induction to nonparticulate antigens in vitro requires the cooperation of T cells and macrophages, but does not occur if the macrophages are allogeneic. The reasons for this were investigated. Malfunction of allogeneic macrophages was excluded by cultures with their syngeneic T cells; suppressor cell induction was excluded by admixture experiments. Thus, T cells and macrophages only cooperated if they were genetically similar. The genetic locus (loci) involved was mapped. Using congenic lines differing only at the H-2 complex, the genetic control of T-macrophage interaction was localized in the H-2 region. Mice with intra H-2 recombinants were used to map the T-macrophage interaction locus in the I-A region of the H-2 complex (formerly known as Ir-I-A) for two antigens, keyhole limpet hemocyanin and poly-L(Tyr, Glu) poly-D,L-ala-poly-L-lys. Recombinants were also used to exclude the presence of another T-macrophage locus in either the K, I-B or I-C, SS-Slp, or D regions of the H-2 complex. Genetic restrictions for T-macrophage interaction in helper cell induction was shown in mice of the H-2k, a° genotypes as well as in H-2
recombinants. The possible mechanisms and significance of this genetic restriction are discussed.

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