# GENETIC CONTROL OF IMMUNOREGULATORY CIRCUITS

## Genes Linked to the Ig Locus Govern Communication between Regulatory T-Cell Sets*

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There is increasing evidence that the intensity and duration of in vitro and in vivo immune response is determined by a series of interactions among different T-cell sets (1). Analysis of one such regulatory circuit has shown that antigen-stimulated Ly1: $Qa1^+$ cells (*a*) induce B cells to secrete antibody, and (*b*) induce a nonimmune set of T-acceptor cells (surface phenotype $Ly123^+Qa1^+$) to participate in the generation of specific suppressive activity (2–4). Because (*a*) the level of suppression that is generated increases in direct proportion to the numbers of antigen-stimulated Ly1-inducer cells and (*b*) one target of Ly123-associated suppression is the T-inducer cell itself (1), this regulatory circuit has been termed feedback inhibition.

The genes which govern this T-T interaction have not yet been identified. There is good evidence that the products of genes linked to the major histocompatibility complex (MHC)[1] play an essential role in certain interactions between immunologic cells (5, 6). Genetic and chemical studies of MHC gene products have also raised the possibility that this gene complex may be homologous to the family of genes that regulate the expression of heavy chains of immunoglobulin (Ig locus), and that the two may share a common evolutionary origin (7–10).

If, indeed, this were the case, products of the two gene complexes might be expected to mediate some overlapping biological functions. We therefore tested the hypothesis that the genes linked to the Ig locus govern the interactions between T-inducer and T-acceptor cells that result in the generation of antigen-specific suppression.

## Materials and Methods

*Mice.* BALB/c mice, 8–12 wk old were obtained from The Jackson Laboratory, Bar Harbor, Maine. CB.20 mice were kindly provided by Dr. M. Potter and Dr. M. Bosma of the National Cancer Institute and the Fox Chase Cancer Institute, respectively. BAB.14 mice were a gift from Dr. M. Bosma.

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[1] *Abbreviations used in this paper:* Ig locus, genes regulating expression of the constant and variable portions of the Ig heavy chain; Ig-$C_H$, genes regulating expression of the constant portions of the Ig heavy chain; MHC, major histocompatibility complex; PFC, plaque-forming cells; SRBC, sheep erythrocytes; $V_H$, variable portion of the Ig heavy chain.



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*Preparation of Lymphocyte Populations.* From the experiment reported in Table I, nylon effluent (11) purified cells and spleen cells were obtained as described previously (12). Controls for the specificity of elimination by Ly antisera were performed as previously described (12). In the experiments reported in Tables II and III, all T cells were prepared from solid phase anti-Ig immunoabsorbents to insure a high degree of purification according to the technique of Wigzell (Table II) (13) and Wysocki and Sato (Table III) (14). In both cases, purified T cells were shown to contain <1% $Ig^+$ cells as judged by immunofluorescence.

*In Vitro Incubation of Lymphoid Populations with Sheep Erythrocytes (SRBC)*

IN VITRO STIMULATION OF LY1 CELLS BY SRBC. $10^7$ highly purified Ly1 cells (see above) were incubated with $2 \times 10^6$ SRBC, according to the method of Eardley and Gershon (11). At the end of 5 d, the remaining viable lymphoid cells were harvested, washed twice, and treated again with anti-Ly2.2 and anti-Ly3.2 + C.

IN VITRO GENERATION OF ANTI-SRBC PLAQUE-FORMING CELLS (PFC). In the experiment described in Table I, $10^7$ spleen cells were stimulated according to the Mishell-Dutton technique as described previously (11). To produce anti-SRBC PFC after stimulation of purified T + B cells (exp. II and III) $2 \times 10^5$ T cells plus $5 \times 10^5$ B cells were incubated with $10^6$ SRBC (without rocking) in Hepes-buffered RPMI (Grand Island Biological Co., Grand Island, N. Y.) plus 10% fetal calf serum (FCS) in an atmosphere of 5% $CO_2$ 95% air at 37°C. All cultures were assayed for PFC after 5 d of culture (11) by the Cunningham modification of the technique originally described by Jerne (11).

## Results

*Genes Linked to the Ig Locus Govern Ly1 Induction of Feedback Suppression (Table I).* We have demonstrated previously that antigen-stimulated Ly1:$Qa1^+$ inducer cells activate a nonimmune set of T-acceptor cells (phenotype $Ly123^+Qa1^+$) to exert substantial suppressive effects (2–4). To determine whether this T-T interaction might be regulated by genes linked to the immunoglobulin heavy chain (Ig locus), Ly1 cells and spleen cells were obtained from either BALB/c or C.B-20 donors, a congenic pair of inbred mice differing only at a cluster of genes linked to the Ig locus (16). Addition of small numbers of SRBC-stimulated BALB/c Ly1 cells to cultures containing BALB/c nonimmune spleen cells (Table I) resulted in substantial inhibition (86–89%) of anti-SRBC PFC formation, but no inhibition of the anti-SRBC response of cultures containing nonimmune C.B-20 spleen cells. By contrast, addition of SRBC-stimulated C.B-20 Ly1 cells to cultures containing nonimmune C.B-20 spleen cells resulted in substantial inhibition (71–76%) of anti-SRBC PFC formation, but no inhibition of anti-SRBC response of cultures containing nonimmune BALB/c T + B cells.

*Gene Products Linked to the Ig Locus Govern the Ability of SRBC-Stimulated Ly1 Cells to Induce Resting T-Cells to Generate Suppression (Table II).* The above results show that the addition of SRBC-stimulated Ly1 cells to cultures containing nonimmune T and B cells inhibited the anti-SRBC PFC response only if the source of Ly1 cells and T + B cells were identical at the Ig locus. We then asked if this genetic restriction governed the interaction between two types of T cells: SRBC-stimulated Ly1 inducer cells and nonimmune acceptor T cells. We found that (Table II) (*a*) Ly1 cells induced suppression in cultures containing nonimmune T + B cells from donors identical at the Ig locus and (*b*) Ly1 cells did not induce suppression in cultures containing T cells from donors that differed at the Ig locus and B cells from donors identical at the Ig locus. These results indicate that the two T-cell sets use gene products that are linked to the Ig locus to effectively interact with each other.

*Genes Regulating Expression of Ig-CH Products (allotypes) Per Se, Do Not Govern Inducer:*

TABLE I

*Gene Products Linked to the Ig Locus Govern Induction of Suppression by T-Cell Sets*

| Source of SRBC stimulated Ly1 cells* | No. Ly1 cells ($\times 10^5$) | SRBC-stimulated lymphocyte cultures ($10^7$ cells/culture) | | |
|---|---|---|---|---|
| | | Source of spleen cells | PFC/culture | Suppression |
| | | | | % |
| — | — | BALB/c | 2,640 ± 60 | Standard |
| BALB/c | 0.7 | BALB/c | 320 ± 26 | 89 |
| BALB/c | 2.0 | BALB/c | 360 ± 20 | 86 |
| C.B-20 | 0.7 | BALB/c | 3,120 ± 80 | 0 |
| C.B-20 | 2.0 | BALB/c | 2,960 ± 204 | 0 |
| — | — | C.B-20 | 2,480 ± 20 | Standard |
| BALB/c | 0.7 | C.B-20 | 3,040 ± 280 | 0 |
| BALB/c | 2.0 | C.B-20 | 2,380 ± 140 | 5 |
| C.B-20 | 0.7 | C.B-20 | 800 ± 60 | 71 |
| C.B-20 | 7.0 | C.B-20 | 600 ± 46 | 76 |

* See text for details of procedure.

TABLE II

*Genes Linked to Ig Locus Control Communication between Inducer and Acceptor T Cells*

| | Source of $10^5$ SRBC-stimulated Ly1 cells | Source of non-immune T cells | Identity at Ig between Ly1 cells and T cells | Suppression |
|---|---|---|---|---|
| | | | | % |
| (a) | — | C.B-20 | | Standard* |
| | C.B-20 | C.B-20 | Yes | 92 |
| | — | BALB/c | | Standard |
| | C.B-20 | BALB/c | No | 2 |
| (b) | — | BALB/c | | Standard‡ |
| | BALB/c | BALB/c | Yes | 98 |
| | — | C.B-20 | | Standard |
| | BALB/c | C.B-20 | No | 0 |

* Source of B cells C.B-20. Mean anti-SRBC PFC responses/$10^6$ cells of SRBC-stimulated cultures containing C.B-20 or BALB/c T cells = 833 ± 160; mean PFC responses of cultures containing purified B cells ± SRBC = 0.

‡ Source of B cells BALB/c. Mean anti-SRBC PFC response/$10^6$ cells of SRBC-stimulated cultures containing C.B-20 or BALB/c T cells = 713 ± 110; mean PFC responses of cultures containing purified B cells ± SRBC = 0.

*Acceptor T-T Interactions (Table III).* The BAB.14 inbred congenic mouse strain, like the C.B-20 congenic strain, expresses Ig-CH gene products of the C57BL/Ka (as defined by anti-allotypic antisera). However, unlike the C.B-20 strain, BAB.14 expresses at least some $V_H$ products of the BALB/c, and is thought to represent a recombinant carrying B6 $C_H$ genes and (mainly) BALB/c $V_H$ genes (17). We therefore asked whether BAB.14 T cells would interact with BALB/c T cells, C.B-20 T cells, or

TABLE III

*Genes Coding for the Constant Portion of the Ig Heavy Chain Do Not Govern Regulatory T-T Interactions*

| Source of $10^5$ SRBC-stimulated Ly1 cells | Source of nonimmune T cells in SRBC-stimulated cultures | Suppression | |
|---|---|---|---|
| | | Exp. 1* | Exp. 2‡ |
| | | % | |
| A. BALB/c | BALB/c | 87 | 77 |
| BALB/c | C.B-20 | 0 | 0 |
| BALB/c | BAB.14 | 79 | 98 |
| B. C.B-20 | BALB/c | 0 | 0 |
| C.B-20 | C.B-20 | 81 | 100 |
| C.B-20 | BAB.14 | 0 | 14 |
| C. BAB.14 | BALB/c | ND | 68 |
| BAB.14 | C.B-20 | ND | 0 |
| BAB.14 | BAB.14 | ND | 100 |

* Source of B cells in all SRBC-stimulated cultures was BAB.14. Anti-SRBC PFC/$10^6$ cells in cultures containing BALB/c T, C.B-20 T, or BAB.14 nonimmune T cells was 480 ± 30, 460 ± 40, and 570 ± 60, respectively. Anti-SRBC PFC/$10^6$ cells in cultures containing SRBC + BAB.14 purified B cells = 0.

‡ Source of B cells in all SRBC-stimulated cultures was the same as the source of nonimmune T cells. Anti-SRBC PFC/$10^6$ cells in cultures containing BALB/c T, C.B-20 T, or BAB.14 nonimmune T cells was 455 ± 15, 560 ± 25, and 575 ± 60. Purified B-cell response in all cultures = 0.

both. We found that small numbers of SRBC-stimulated BALB/c Ly1 cells induced substantial (80–90%) suppression of the anti-SRBC response in cultures containing BALB/c or BAB.14 nonimmune T cells, but not in cultures containing C.B-20 T cells. These findings indicate that identity at Ig-$C_H$ locus, per se, does not allow efficient T-T interactions. This is further shown by the finding that C.B-20 Ly1 cells induced high levels of suppression in cultures containing C.B-20 nonimmune T cells but not BALB/c or BAB.14 nonimmune T cells. Taken together, these findings indicate that identity at the Ig locus is required for efficient inducer:acceptor T cell communication and this does not reflect Ig-$C_H$ genes per se.

An important point also comes from the data shown in experiment 1 (Table III). These results confirm the conclusions drawn from results in Table II: Communication between T-inducer and T-acceptor cells requires that both T-cell sets express identical Ig-linked genes. In addition, it shows that this genetically restricted communication between T-cell sets is independent of the Ig-phenotype of B cells in the assay cultures. Thus, Ig-restricted T-T communication reflects gene products intrinsic to the relevant T-cell sets and cannot be accounted for by, for example, passively acquired Ig-linked products produced by B cells in assay cultures.

## Discussion

The results we have presented demonstrate the ability of antigen-stimulated Ly1 cells to induce a nonimmune set of T-acceptor cells (phenotype $Ly123^+Qa1^+$) (2–4) and requires that both cell sets share genes that are linked to the Ig locus and that this

requirement is independent of the Ig-linked gene products expressed on the interacting B cells. The most obvious interpretation of this is that products of Ig-linked genes govern interactions between T-inducer and T-acceptor cell sets that generate antigen-specific suppression. The relevant genes do not code for Ig-$C_H$ structures per se (Table III); they may code for $V_H$ structures or, as yet, unidentified cell surface molecules.

The experiments of Binz and Wigzell (18) and of Krawinkel et al. (19) have indicated that at least some sets of T cells carry at their membrane surface products of Ig-linked genes. These molecules resemble the variable portion of immunoglobulin heavy ($V_H$) chains and may permit highly specific cell interactions that regulate the production of antibodies carrying well-defined idiotypic markers (20, 21). Possibly, Ly1-induction of resting T cells to generate specific T suppression is governed by analogous or identical Ig-linked gene products.

Genes coding for minor histocompatibility antigens may also be linked to the Ig locus (22, 23) and therefore represent additional candidates that may participate in interactions between inducer and acceptor T cells. If so, there influence cannot be attributed to standard allogeneic effects (5, 6) that might override suppression, because mixtures of T and B cells (Table II) or immune Ly1 cells and B cells (Table III) that differ at the Ig locus do not stimulate positive allogeneic effects in vitro. Further, the results presented in Table III (exp. 1) show that the structures that govern this interaction are not expressed on B cells. One intriguing explanation for this observation is that genes coding for $V_H$-like structures expressed on T cells are not identical to those controlling expression of $V_H$ structures on B lymphocytes. However, there are other equally plausable explanations for this observation and further analysis is necessary to address this point directly.

In any event, the failure of Ly1 cells to induce Ig-disparate T cells to suppress does not reflect a positive allogeneic effect, nor the influence of minor histocompatibility antigens because they are selectively expressed on T cells (and thus would be, by definition, differentiation antigens rather than minor histocompatibility antigens). Also relevant to this question is the observation that Ly1 cells from BAB.14 mice (which differ from both C.B-20 and BALB/c mice at minor histocompatibility loci (23)) interact efficiently with BALB/c T cells but not at all with C.B-20 T cells.

For these and other reasons, we think that the Ig-linked genes that govern communication between inducer and acceptor regulatory T cells, code for or control the expression of $V_H$-like surface structures. Further characterization of Ig-linked gene products that govern interactions between inducer and acceptor T cells include more precise genetic mapping using Ig-congenic recombinant and $F_1$ mouse strains and analysis of the inhibitory effect of various serologic reagents on T inducer: acceptor interactions.

Regardless of the precise molecular nature of these gene products, the observations that: (*a*) They represent polymorphic variants of Ig-linked genes and (*b*) they control communication among some sets of immunocompetent cells, have several important implications. Because some MHC-linked gene products (Ir genes) also influence communication among immunological cells, it is likely that analogous immunologic misunderstandings that contribute to Ir-gene defects and immunologic diseases will be associated with particular Ig-linked polymorphic determinants. It is therefore essential to find out whether inducer:acceptor communication governed by Ig-linked gene products is also influenced by MHC gene products. If so, it is likely that the

degree of correlation between some human diseases and genetic polymorphisms might be improved considerably by coordinate analysis of both MHC and Ig haplotypes of the relevant patient populations.

## Summary

Antigen-stimulated Ly1:Qa1$^+$ cells induce a nonimmune set of T-acceptor cells (surface phenotype Ly123$^+$Qa1$^+$) to participate in the generation of specific suppressive activity. The experiments reported here were designed to test the possibility that the interaction between T-inducer and T-acceptor cells might be governed by genes linked to the Ig locus. We find that inducer:acceptor interactions occur only if the inducer and acceptor T-cell sets are obtained from donors that are identical at the Ig locus and are independent of the Ig locus expressed on the B cells used for assay of T-helper activity. In addition, experiments using inducer and acceptor T cells from the congenic recombinant BAB.14 strain show that T-T interactions are not governed by Ig-$C_H$ genes, per se. These data indicate that T-inducer:T-acceptor interactions are governed by Ig-linked genes that may control expression of $V_H$-like structures on T cells, or control expression of as yet unidentified cell-surface molecules.

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