RESPONDER T CELLS DEPLETED OF ALLOREACTIVE CELLS REACT TO ANTIGEN PRESENTED ON ALLOGENEIC MACROPHAGES FROM NONRESPONDER STRAINS*

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The immune response (*Ir*) gene-controlled nonresponsiveness of inbred mouse strains to certain antigens is the result of a failure of T cells to recognize antigen in the context of self major histocompatibility complex (MHC) molecules. This conclusion is based on two types of experimental evidence. First, it has been demonstrated that T cells from (responder × nonresponder)F_1 animals are activated by antigen on antigen-presenting cells (henceforth referred to as macrophages) from the responder parent, but do not react to antigen on macrophages from the nonresponder parent (reviewed in ref. 1). Second, T cells that are genetically nonresponders but have matured in a (responder × nonresponder)F_1 environment have been shown to become responders, but only to the antigen presented by cells of the responder parent (2–8). Although this failure of interaction between T cells and nonresponder macrophages has been interpreted as evidence that nonresponder macrophages fail to form immunogenic complexes of antigen and MHC molecules (1, 9), another explanation of these data is also possible, namely that nonresponder strains lack T cells with specificity for the particular combination of antigen with MHC molecules (3).

In an attempt to distinguish between these two possibilities, we have developed an assay that measures *Ir* gene-controlled proliferation of T cells in response to antigens presented by allogeneic macrophages. We describe here the assay system, and provide evidence for unimpaired antigen presentation by nonresponder macrophages.

Materials and Methods

Mice. All mouse strains were obtained from our colony at the Max-Planck-Institute for Biology. 8–16-wk-old female and male mice were used.

Antigens. The random co-polymer of amino acids, poly(Glu^{40} Ala^{60}) (GA) was a gift from Dr. P. H. Maurer (Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pa.). The antigen (dissolved in distilled water, pH 8.1) was diluted to a concentration of 0.2–0.4 mg/ml in RPMI 1640 (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.), and sterilized by γ-irradiation (3,000 rad). Lactate dehydrogenase B4 (LDH$_B$) (Boehringer, Mannheim, F.R.G.) was dialyzed against culture medium, sterilized, and stored at 4°C.

Monoclonal Antibodies. Ascites fluid containing high titered monoclonal antibodies was produced using the hybridomas B15-124R$_1$ (anti-Ia.m2) (10), and P47-42 (anti-Ia.m9) (11).

Cell Preparation.   The culture medium used thoughout was RPMI 1640 supplemented with 5% heat-inactivated horse serum (Gibco Laboratories), antibiotics, and 5×10^{-5} M 2-mercaptoethanol. T cells were prepared from nucleated spleen cells of unprimed mice by nylon wool column separation. Macrophages were obtained by culturing cells from peritoneal washing in 50-mm diameter glass dishes (1×10^7 cells/ml, 2 ml/dish) for 8 h. Nonadherent cells were then decanted, and adherent cells (~30% of input) were harvested using a rubber policeman.

Removal of Alloreactive T Cells.   The method described by Thomas and Shevach (12) was employed with slight modifications. T cells (4×10^7) were cultured with 1×10^7 allogeneic macrophages in 20 ml medium. After 48 h of culture, 2 µg/ml 5-bromo-2'-deoxyuridine (BudR) (Sigma Chemical Co., St. Louis, Mo.) was added, and after 72 h the cultures were illuminated for 90 min to eliminate cells synthesizing DNA.

In Vitro Priming of T Cells.   T cells (1.5×10^7) depleted of alloreactive cells, and allogeneic macrophages (5×10^6) were cultured in 20 ml medium containing 40 µg/ml of GA for 3 d. Alternatively, T cells were cultured with macrophages pulsed previously with antigen ($1 \times 10^7/$ml of macrophages incubated with 80 µg/ml of GA for 60 min at 37°C). Both procedures resulted in equally efficient priming. After 3 d, the cells were washed, adjusted to the initial concentration, and cultured for 4 d in fresh medium without antigen. At the end of the 7-d culture period, ~10% of input T cells were recovered.

Secondary Cultures.   T cells decanted from priming cultures were distributed in flat-bottomed microculture plates (Falcon Labware, Oxnard, Calif.) at a density of 1×10^5 per well, together with 1×10^5 fresh macrophages, with or without antigen. To exclude the possibility that contaminating T cells in the macrophage populations may contribute to the response, priming cultures contained macrophages only, and freshly prepared macrophages were tested for proliferation to GA. Such cultures were regularly negative in terms of proliferation. Proliferation in secondary cultures was measured by incorporation of [^{3}H]thymidine on day 3 (13). Blocking of T cell proliferation with monoclonal Ia antibodies was performed as previously described (13).

Results

As illustrated in Fig. 1, T cells depleted of alloreactive cells, and subsequently primed in vitro with GA on allogeneic macrophages, were capable of mounting a secondary proliferative response to GA presented by allogeneic macrophages. No response was obtained to either the same antigen on syngeneic macrophages, or an unrelated antigen, LDH$_B$, on allogeneic macrophages. The proliferation was blocked by a monclonal Ia antibody (anti-Ia.m9) against the A molecule of the macrophage, but was not affected by anti-Ia.m2 recognizing the A molecule controlled by the T cells' haplotype. Thus, this type of response is antigen specific and involves the recognition of Ia molecules of the allogeneic macrophage.

We have recently demonstrated that unprimed lymph node T cells carrying *H-2* haplotypes *p*, *q*, *r*, *s*, *u*, and *v* are low responders or nonresponders to GA (13; and N. Ishii, unpublished results). To clarify whether the nonresponsiveness was due to a failure of antigen presentation, we tested a panel of nonresponder macrophages for their capacity to induce anti-GA response of allogeneic T cells from responder strains. The experiments in Table I demonstrate that macrophages from nonresponder *H-2* haplotypes *p*, *q*, *r*, *s*, and *u* are all capable of presenting GA to T cells carrying responder haplotypes *b* and *d*. T cell responses were observed to two antigens (GA and LDH$_B$) tested so far, in several different responder-nonresponder combinations (N. Ishii, unpublished data).

Discussion

Two important conclusions follow from the results presented in this communication: first, the data confirm the existence in normal, unprimed mice of T cells capable of
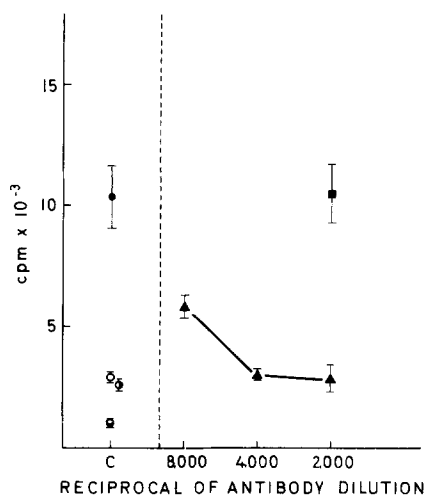
FIG. 1.   Proliferation of CBA (responder) T cells depleted of alloreactivity against H-2^q, in response to GA presented by B10.Q (nonresponder) macrophages (●). Antibody against Ia antigen on macrophages (▲: anti-Ia.m9), but not that against Ia antigen of the T cells' haplotype (■: anti-Ia.m2), inhibits T cell proliferation. The control cultures (C) include CBA T cells with either B10.Q macrophages without antigen (○), or B10.Q macrophages with LDH$_B$ (◒), or CBA macrophages with GA (◓).

TABLE I

T Cells from Responder Strains B10 and B10.D2 Proliferate in Response to GA Presented on Macrophages from Nonresponder Strains

Macrophages	Antigen	In vitro secondary proliferative response (cpm ± SD) by T cells depleted of alloreactivity against macrophage MHC antigens	
		B10 T cells	B10.D2 T cells
B10.P	GA	30,440 ± 2,213	11,673 ± 2,469
B10.P	—	556 ± 24	523 ± 180
B10.Q	GA	37,777 ± 87	32,967 ± 4,280
B10.Q	—	1,413 ± 27	782 ± 186
B10.RIII	GA	29,520 ± 3,673	40,394 ± 6,917
B10.RIII	—	1,513 ± 199	2,160 ± 243
B10.S	GA	10,102 ± 771	20,783 ± 4,023
B10.S	—	1,827 ± 89	3,421 ± 520
B10.PL	GA	31,529 ± 2,486	17,603 ± 1,777
B10.PL	—	778 ± 298	732 ± 62

recognizing antigen in the context of allogeneic MHC molecules (12, 14); and second, because several different nonresponder macrophages are capable of presenting antigen to T cells carrying different H-2 haplotypes (except the syngeneic one), the mechanism of nonresponsiveness cannot be a failure of antigen presentation. Our observation also renders a determinant-selection mechanism (reviewed in ref. 1) operating at the level of macrophages unlikely. It is in fact difficult to envisage that macrophages from several nonresponder strains all present an immunogenic determinant to T cells from a particular allogeneic strain, and fail to do so to T cells of their own haplotype. The counterargument that all these macrophages might present different determinants of the same antigen to different T cell clones, each restricted by a different allogeneic MHC molecule, cannot be excluded by these experiments. However, such an argument would shift the cause of nonresponsiveness from macrophages to the T cell

repertoire, and would thus be consistent with our main conclusion that nonrespon-siveness cannot be a result of defective antigen presentation. At any rate, the important implication of our data is that the rules of Ir gene control established in syngeneic and semisyngeneic systems do not apply to the interaction of T cells with allogeneic macrophages.

Our results conflict with those of some authors who could not detect T cells restricted by allogeneic MHC antigens in normal animals (15, 16). It is not clear whether this discrepancy results from differences in the experimental systems used, or from a low frequency of cells restricted by allogeneic MHC antigens in the peripheral T cell pool. Comparative studies on precursor frequencies of T cells restricted by self and allogeneic MHC antigens, respectively, may help to resolve this conflict. Further studies are also required to clarify whether T cells recognizing foreign antigen in the context of allogeneic MHC antigens represent a part of the T cell repertoire that is useless for the individual, or whether allogeneic restriction results from cross-reactivity of T cells normally recognizing another foreign antigen together with self MHC molecules.

Summary

T cells from strains responder to the antigen poly($Glu^{40}Ala^{60}$) (GA) were depleted of alloreactive cells by bromo-deoxyuridine and light treatment, and were subse-quently primed in vitro with GA presented by allogeneic macrophages from nonre-sponder strains. Antigen-specific secondary proliferative responses restricted by allo-geneic Ia molecules of the macrophages were obtained in all strain combinations tested. These data indicate that Ir gene-controlled nonresponsiveness cannot be the result of a failure of antigen presentation.

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