ACTIVATION OF T AND B LYMPHOCYTES IN VITRO

III. Presence of Ia Determinants on Allogeneic Effect Factor*

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Previous studies from our laboratory have focused on the biological and biochemical properties of an active product obtained from populations of activated T lymphocytes during short-term in vitro reactions with foreign alloantigens (1). The active product of supernates from such cultures, which we have termed allogeneic effect factor (AEF), was shown to be capable of acting directly on B lymphocytes, in the presence of antigen, to effect triggering and subsequent differentiation and proliferation to antibody-forming cells in vitro. The chromatographically purified active fraction of AEF was found to be a protein moiety of 30,000–40,000 daltons (1). The active molecule(s), although not manifesting specificity for antigens against which the in vitro antibody responses were directed, did exhibit some strain-specific properties suggesting a relationship to antigens or gene products coded in the major histocompatibility gene complex.

Recently, investigations in several laboratories using antilymphoid cell antisera prepared between recombinant mice differing at genes present in the I region of the H-2 complex identified a new antigen system, which has been termed Ia, coded for by genes in the I region; the Ia antigens have been found to exist predominantly on B cells and macrophages and to varying extents on T cells (reviewed in ref. 2). Accordingly, the possibility must be considered that gene products in this region may be involved in regulatory cell interactions in immune responses. The experiments presented in this paper demonstrate that the active enhancing factor(s) in AEF can be removed by an immunoabsorbant prepared with an anti-Ia antiserum indicating that, indeed, the biologically active moieties responsible for T-B cell interactions are probably products of genes in the I region of the H-2 gene complex.

Materials and Methods

The tissue culture system and the assay for in vitro antibody responses to sheep erythrocytes (SRBC), the preparation of anti-δ serum, and the method employed for depletion of T lymphocytes are described elsewhere (1, 3). Inbred DBA/2, (C3H × DBA/2)F1, (C3D2F1), (C57BL/6 × A)F1, (B6AF1), C57BL/10 (B10) and congenic B10.A and B10.D2 mice were obtained from Jackson Laboratories, Bar Harbor, Maine. The AEF employed in this study was prepared from DBA/2 (H-2d) thymocytes activated against C3H (H-2a) histocompatibility antigens, as previously described (1).

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The alloantisera (or Ehrlich's ascites from the same donor animals) employed were prepared and tested for serological reactivity as described in detail previously by Sachs and Cone (4). The following antisera were used: (a) B10.A anti-B10—this antiserum contains antibodies directed against antigens coded for by the H-2\(^b\) gene complex; it cross-reacts with antigens coded by genes in the I region of H-2\(^d\) (Ia.8) but not with antigens coded by genes in either the K or D regions of H-2\(^d\); (b) (B6A)\(F_1\) anti-B10.D2—this antiserum contains predominantly antibodies reactive with specificity H-2.31 present on cells from animals with the H-2\(^d\) allele; it does not contain anti-Ia.8 activity. Although at the initiation of these studies there were no known antibodies reactive with the Ia\(^d\) specificities in this antiserum, subsequent analyses have demonstrated the presence of antibodies reactive with a new Ia specificity present also in H-2\(^d\) (5). Polyvalent rabbit antimouse immunoglobulin (Ig) antisera were prepared as described previously (6).

Immunoadsorbents were prepared by the method of Avrameas and Ternyck (7) by direct polymerization of immune or normal sera with glutaraldehyde at pH 5.0. Unfractionated AEF were absorbed either (a) directly with excess immunoadsorbents prepared from alloantisera or control normal sera in a batchwise procedure, or (b) indirectly by incubation first with the alloantisera or normal sera (at a ratio of 6 parts AEF to 1 part serum) for 1 h at room temperature and the absorption of the resulting complex with a rabbit antimouse Ig immunoadsorbent. The absorbed AEF were assayed for biological activity as 1:10 (direct absorption) or 1:15 (indirect absorption) dilutions after filtration through 0.45 \(\mu\) Millipore filters (Millipore Corp., Bedford, Mass.).

**Results**

Two types of experiments utilizing the AEF prepared from DBA/2 (H-2\(^d\)) T cells will be presented in this study. In the first (Fig. 1), three different concentrations of AEF were directly absorbed independently by immunoadsorbents prepared from (B6A)\(F_1\) anti-B10.D2 and B10.A anti-B10 alloantisera and by an adsorbent prepared from normal B10.A serum. These AEF were then compared to unabsorbed AEF for biological activity on the in vitro response to SRBC of DBA/2 B lymphocytes. As shown in Fig. 1, cultures of untreated control whole spleen cells developed primary IgG anti-SRBC responses of around 1,200 PFC; anti-\(\theta\)-treatment diminished this response to around 150 PFC. The addition of unabsorbed AEF to such anti-\(\theta\) serum-treated B cells reconstituted and enriched the response markedly and in a dose-related manner at all three concentrations of AEF employed. The AEF subjected to the adsorbent prepared from normal B10.A serum exhibited virtually identical biological activity. The AEF obtained from the immunoadsorbent prepared from (B6A)\(F_1\) anti-B10.D2 serum retained essentially normal biological activity at the highest concentration (1:5), but the lowest concentration subjected to absorption (1:20) was around 45% lower in activity than the normal serum control. The AEF subjected to the B10.A anti-B10 (anti-\(\lambda\)) immunoadsorbent, on the other hand, exhibited markedly diminished (80% or more) activity at all three concentrations indicating substantial reactivity of this antiserum with the biologically active component(s) of AEF.

The second type of experiment was a modification of the preceding one in that an indirect absorption procedure was employed. Thus, AEF was reacted first with either of the two alloantisera or normal serum and the mixture was subsequently subjected to an immunoadsorbent prepared from polyvalent rabbit antimouse Ig antiserum. The results of this experiment, shown in Table 1, corroborate the results of the first experiment. Thus, anti-\(\theta\) serum-treated DBA/2
spleen cells, which fail to respond to SRBC in culture in the absence of AEF, (cf. cultures I and II) are reconstituted in their responsiveness when AEF is added to the culture (III). In this experiment, AEF activity was not affected by exposure to either normal mouse serum or the (B6A)F₂ anti-B10.D2 antiseraum followed by absorption by the antimouse Ig adsorbent (cultures IV and V) whereas the B10.A anti-B10 sera completely removed all biological activity (culture VI).

Discussion and Summary

Observations from our own laboratories, as well as those of others, have demonstrated the critical role of histocompatibility gene products in governing the cell-cell interactions concerned with development and regulation of immune responses in several species (8-12). In mice, the relevant genes concerned have been shown to be located in the K end of the H-2 complex, i.e. in the K and/or I

![Diagram](image-url)
**Biological Activity of AEF on In Vitro Responses of DBA/2 B Lymphocytes after Indirect Absorption by Alloantiserum and an Antimouse Immunoglobulin Immunoadsorbent**

| Culture | Cultured DBA/2 cells | AEF | Antiserum treatment | Anti-SRBC response $\dagger$ |
|---------|----------------------|-----|---------------------|-----------------------------|
| I       | Whole spleen         | -   | -                   | 690                         |
| II      | Anti-8 serum-treated spleen | -   | -                   | 20                          |
| III     | "                    | +   | -                   | 840                         |
| IV      | "                    | +   | Normal mouse serum  | 660                         |
| V       | "                    | +   | (B6A)F, anti-B10.D2 | 740                         |
| VI      | "                    | +   | B10.A anti-B10      | 0                           |

* DBA/2 whole spleen or anti-8 serum-treated spleen cells were cultured either alone or with unabsorbed or absorbed AEF (1:15) in the absence or presence of SRBC.

† AEF was incubated for 1 h at room temperature with the sera indicated and then the mixture was subjected to an immunoadsorbent prepared from rabbit antimouse immunoglobulin antiserum.

§ IgG PFC per 10$^7$ cultured cells.

regions (13, 14). These discoveries have placed histocompatibility gene products on a more complex level of biologic function than was heretofore generally considered (15). Thus, the hypothesis was made from these observations that genes in the $H-2$ complex coded for products involved in the development of effective cell-cell interactions in the immune response (8, 9, 15). The recent identification of cell surface macromolecules on lymphocytes and macrophages, that may be distinct from immune response gene products but are likewise coded for by genes in the I region, has provided a group of suitable candidate molecules for such a role (2).

In our initial studies on the biological and biochemical characteristics of AEF, we were impressed by the apparent preferential activity of the highly purified AEF preparations on B lymphocytes syngeneic to the activated T-cell population from which the AEF was obtained (1). Since a prediction of the aforementioned hypothesis is, of course, that the active molecules involved in regulatory immunocompetent cell interactions are gene products of the $H-2$ complex, and, accordingly, should be reactive with antisera directed against components of this complex, we were prompted to perform the appropriate analyses on our preparation of AEF.

The experiments presented here demonstrate that the enhancing activity of AEF obtained from T cells of the $H-2^d$ haplotype can be specifically removed by immunoadsorbents prepared from antisera reactive with Ia molecules of the $H-2^d$ allele. Identical results were obtained in experiments with both direct and indirect absorption procedures. The possibility that the reaction of AEF with the B10.A anti-B10 (anti-Ia.8) antiserum resulted in release of some components that were in turn toxic to the cultured cells, has been made unlikely in these studies by the use of a direct adsorption method utilizing an immunoadsorbent prepared from thoroughly washed glutaraldehyde-linked antibodies.

The results obtained with the (B6A)F, anti-B10.D2 antiserum deserve some comment. This antiserum contains antibodies directed predominantly against
the H-2K region specificity, H-2.31, but may also be reactive with recently determined Ia\(^a\) specificities (5). The capacity of this antiserum to directly absorb approximately 45% of the AEF activity at the lowest concentration of AEF employed (Fig. 1) could be interpreted to indicate the reactivity of AEF with anti-H-2K antibodies. However, the data presented here are also consistent with the interpretation that partial adsorption by the direct immunoadsorbent and lack of adsorption by the indirect method (in which only a high concentration of AEF was incubated with the alloantisera) reflect reactivity of AEF with anti-Ia\(^a\) antibodies present in this antiserum.

We conclude, therefore, that the biologically active enhancing moieties of AEF bear Ia determinants and therefore are most probably gene products of the I region of the H-2 gene complex. Recent data from other investigators have shown that an antigen-specific T-cell product could be specifically adsorbed by immunoadsorbents prepared from antisera directed against the K end of H-2 (16). Since the latter antisera may contain antibodies reactive with specificities of both K and I regions, it is possible that the use of selective anti-Ia sera may yield results consistent with those presented here. Taken collectively, these observations indicate that I-region gene products may be intimately involved in the mechanism of cell-cell interactions and responsible for the regulation of immune responses.

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