IN VITRO ANALYSIS OF ALLOGENEIC LYMPHOCYTE INTERACTION

II. *I*-Region Control of the Activity of a B-Cell-Derived *H-2*-Restricted Allogeneic Effect Factor and its Receptor during B-Cell Activation*

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On the basis of studies with immunoadsorbent columns, many soluble, lymphocyte-derived, helper and suppressor factors, which mediate T-cell B-cell macrophage interaction during antibody synthesis, have been shown to consist of *H-2*-linked *I*-region associated (Ia)[1] antigens (1–3). It has been postulated that *I*-region genes also determine the putative receptors for both helper (4–6) and suppressor (7–10) factors. Both helper factors and their receptors have been reported to be controlled by the *I-A* subregion (4, 5, 10). Suppressor cell factors and their receptors have been found to be products of either the *I-J* subregion (7, 10) or the *I-C* subregion (9).

We have previously demonstrated that an antigen-nonspecific helper factor, allogeneic effect factor (AEF), is comprised of Ia antigens derived from both the activated responder and irradiated stimulator spleen cells of a mixed lymphocyte culture reaction (MLR) (11). This AEF helped a primary and secondary antibody response of both T-cell-depleted responder spleen B cells and stimulator spleen B cells. Identity in the *I-A* and/or *I-B* subregions was required for the interaction between this AEF and primary (nonimmune) spleen B cells.

An AEF produced by Ia-negative activated responder cells and irradiated T-cell-depleted stimulator cells helped a secondary antibody response of T-cell-depleted stimulator B cells but not responder B cells (12). This genetically restricted AEF was shown to contain Ia antigens determined by the stimulator haplotype but not the responder haplotype. These observations indicated that restricted AEF is the product of a stimulator B cell and/or macrophage.

In this report, we show that restricted AEF is a B-cell-derived helper factor which consists in part of active Ia antigen components determined by the *I-A* subregion. We also demonstrate that restricted AEF possesses a target B-cell receptor which may also be controlled by the *I-A* subregion. Taken together, the data suggest that the *H-2* restriction in the activity of restricted AEF

* Supported by the Medical Research Council of Canada grant MA 5729.

[1] *Abbreviations used in this paper:* AEF, allogeneic effect factor; DNP, 2,4-dinitrophenyl; FCS, fetal calf serum; I, immune response; Ia, *I*-region associated; KLH, keyhole limpet hemocyanin; MLR, mixed lymphocyte culture reaction; NSE, nonspecific esterase; PFC, plaque-forming cell; RBC, erythrocytes; TNP, 2,4,6-trinitrophenyl.

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TABLE I
H-2 Haplotype Origin of Strains Used

Strain	Haplotype	Region*								
			I							
		K	*A*	*B*	*J*	*E*	*C*	*S*	*G*	*D*
B10.A	*a*	*k*	*k*	*k*	*k*	*k*	*k/d*‡	*d*	*d*	*d*
B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
A.TL	*t1*	*s*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*d*
A.TH, B10.S(7R)	*t2*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*d*
B10.HTT	*t3*	*s*	*s*	*s*	*s*	*k*	*k*	*k*	*k*	*d*
B10.S(9R)	*t4*	*s*	*s*	?	*k*	*k*	*d*	*d*	*d*	*d*
A.TFR5	*ap5*	*f*	*f*	*f*	*f*	*f*	*k*	*k*	*k*	*d*

* Haplotype origin of *H-2* regions according to references 21 and 39.

‡ It is not yet certain whether the *I-C* subregion of the $H\text{-}2^a$ haplotype is derived from the $H\text{-}2^k$ or $H\text{-}2^d$ haplotype.

during B-cell activation is mediated, in part, by a recognition of complementarity between Ia antigens in this factor and *I*-region products in its receptor. A preliminary report of some of the data included here has been previously published (13).

Materials and Methods

Mice. All inbred strains of mice used in this study were maintained in our colony at the University of Toronto. These strains were derived from breeding pairs kindly supplied by Doctors H. O. McDevitt, Stanford University, Stanford, Calif., and D. C. Schreffler, Washington University, St. Louis, Mo.

Antisera. The antisera used in these studies and the respective *H-2* regions or subregions immunized against are as follows: A.TH anti-A.TL (anti-I^k, S^k, G^k); A.TL anti-A.TH (anti-I^s, S^s, G^s); (B10.A × A.TL)F_1 anti-B10.S(9R) (anti-$I\text{-}A^s$, $I\text{-}B^s$?); (B10.A × A.TL)F_1 anti-B10.HTT (anti-$I\text{-}A^s$, $I\text{-}B^s$, $I\text{-}J^s$) B10.HTT anti-B10.S(7R) (anti-$I\text{-}E^s$, $I\text{-}C^s$, S^s, G^s); and (B10.HTT × A.TFR5)F_1 anti-A.TH (anti-$I\text{-}E^s$, $I\text{-}C^s$, S^s, G^s). The latter three sera were a kind gift from Doctors D. B. Murphy and H. O. McDevitt, Stanford University, Stanford, Calif. All of the above sera were raised by hyperimmunization of recipient mice with donor spleen and lymph node lymphocytes as previously described (14). An AKR/J anti-AKR/Cum anti-Thy-1.2 serum, produced by using donor thymocytes and spleen cells for immunization, was used to deplete spleen cell suspensions of T cells. The *H-2* haplotypes of some of the strains used in this study are shown in Table I.

Antigens and Immunizations. The preparation of the 2,4-dinitrophenyl (DNP) keyhole limpet hemocyanin (KLH; Calbiochem, Downsview, Ontario) conjugate, DNP_{11}-KLH (per 10^5 daltons), and the immunization of mice with this antigen were performed as previously reported (12). Mice were sacrificed 4–8 wk postimmunization and were used as primed spleen cell donors.

Preparation of Restricted AEF. Restricted AEF was prepared as previously described (12). Briefly, as shown in Fig. 1, B10.BR($H\text{-}2^k$) lymphocytes were allogeneically activated against $H\text{-}2^s$ antigens by the intravenous injection of irradiated (800 rads) B10.S ($H\text{-}2^s$) recipients with 1–1.5 × 10^8 B10.BR donor thymocytes. 5 days later, the activated responder B10.BR cells were harvested from the stimulator B10.S recipients. Ia-negative responder cells were prepared by treatment of 10^8 cells with 1 ml of A.TH anti-A.TL diluted 1:5 for 20 min at room temperature. Cells were then centrifuged and resuspended in 3 ml of agarose-adsorbed rabbit complement diluted 1:9 and incubated for a further 45 min at 37°C, centrifuged, washed twice, and resuspended in serum-free Click's medium (15) supplemented with 4 mM glutamine and 5×10^{-5} M 2-mercaptoethanol before

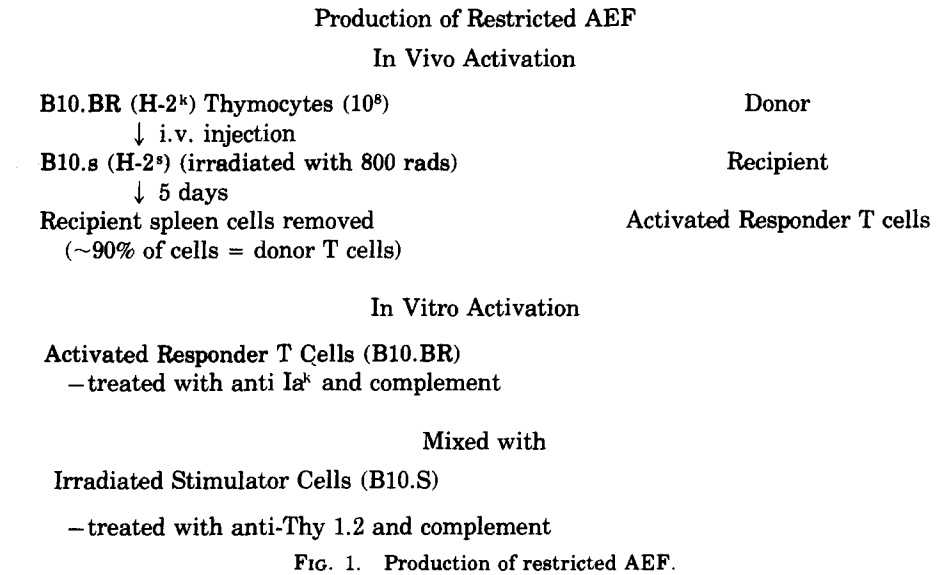

FIG. 1. Production of restricted AEF.

culture. T-cell-depleted (<3% T cells, [12]) normal stimulator B10.S cells were similarly prepared by treatment with anti-Thy-1.2 (AKR/J anti-AKR/Cum) and rabbit complement. Restricted AEF was prepared by collection of supernates from 16–24 h MLR cultures between 10^7 Ia-negative activated responder B10.BR cells and 10^7 normal irradiated (3,000 rads) T-cell-depleted stimulator B10.S spleen cells. Culture conditions and medium were the same as reported earlier (12).

Immunoadsorption and Assay of Restricted AEF Activity. The methods used for the immunoadsorption on antibody-coated columns and assay of restricted AEF activity were previously described (12). The adsorption of restricted AEF activity was carried out during a 1 h incubation at 4°C on immunoadsorbents each consisting of approximately 5 mg of the IgG fraction a given mouse anti-Ia serum coupled to 1 g of cyanogen-bromide-activated Sepharose 4B (Pharmacia Fine Chemicals Inc., Dorval, Quebec). The activities of various column effluent fractions were then tested.

The cellular expression of a receptor for restricted AEF was analyzed by adsorption (9) of its activity by various cell types. All cell suspensions were prepared in serum-free Click's medium and were washed twice with this medium before adsorption. They were shown to contain greater than 90% viable cells as judged by trypan blue dye exclusion. The adsorbing cells used, derived from normal unprimed B10.S donors, were thymocytes, lymph node cells, spleen cells, spleen T cells prepared by passage through a nylon wool column (16), T-cell-depleted spleen cells prepared by treatment with anti-Thy-1.2 plus complement, Ia-negative T-cell-depleted spleen cells, and macrophage-depleted T-cell-depleted spleen cells (prepared as outlined in the section below). To prepare Ia-negative T-cell-depleted spleen cells, approximately 7.5×10^7 T-cell-depleted cells were washed twice, fractionated on a 1 g fetal calf serum (FCS) gradient to remove dead cells (17), washed twice in serum-free medium, treated with anti-Ia serum plus complement (see above), washed twice, and then used for adsorption. Approximately 10^7 cells were recovered from the original T-cell-depleted spleen cell population after this treatment. 1 ml of restricted AEF was incubated in undiluted form with $0.1–1.0 \times 10^8$ adsorbing cells in serum-free Click's medium at 4°C for 30 min with occasional mixing. After centrifugation of the cells, the activity of nonadsorbed AEF in the supernates was tested.

The helper activity of either unadsorbed or adsorbed (as above) restricted AEF was assayed in an in vitro secondary anti-DNP plaque-forming cell (PFC) response of DNP_{11}-KLH primed T-cell-depleted B10.S spleen cells after a 6 day culture in Click's medium containing 5% heat-inactivated FCS (Click-FCS). Anti-DNP indirect (IgG) PFC were evaluated in triplicate 0.3-ml cultures, containing 10^6 spleen cells, by the Cunningham and Szenberg method (18) by using 2,4,6-trinitrophenyl (TNP)-coupled burro erythrocytes (RBC) as indicator cells.

Depletion of Macrophages from Spleen Cell Suspensions. The phagocytic cells present in a T-cell-depleted spleen cell population were removed by the carbonyl-iron technique as previously reported (19). To a suspension of cells at 20 × 10^6/ml in Click-FCS was added powdered iron (Fisher Scientific Co., Don Mills, Ontario) at a concentration of 50 mg/ml. This suspension was incubated for 1 min at 37°C, then layered over an equal vol (5 ml) of Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc., Dorval, Quebec and Winthrop Laboratories, Aurora, Ontario) and centrifuged at 450 *g* for 45 min. The cells banding at the Click-Ficoll interface were aspirated with a Pasteur pipette and washed twice with serum-free Click's medium before use. The extent of contamination with monocytes and/or granulocytes was determined cytochemically by the nonspecific esterase (NSE) staining method of Yam et al. (20).

Application of this technique to T-depleted spleen cells reduced the percentage of NSE-stained cells from approximately 5% to less than 1%. Thus, the technique was adopted to remove macrophages from T-depleted spleen cells used for the stimulator cell population in an MLR to generate restricted AEF and for the cellular adsorption of restricted AEF activity.

Results

I-Region Control of Restricted AEF Activity. By the use of appropriate immunoadsorbents, restricted AEF was previously shown to consist of Ia antigens derived only from the stimulator (H-2^s) and not responder (H-2^k) haplotype (12). The anti-Ia antiserum used for adsorption was A.TL anti-A.TH (anti-I^s, S^s, G^s), which potentially contains antibody activity against determinants of the entire *I*-region. It was therefore of interest to ascertain whether a specific I^s-subregion (s) codes for the Ia antigens present in restricted AEF.

Restricted AEF was fractionated on immunoadsorbents conjugated with either A.TL anti-A.TH (anti-I^s, S^s, G^s), (B10.A × A.TL)F_1 anti-B10.S(9R) (anti-I-A^s, I-B^s?), (B10.A × A.TL)F_1 anti-B10.HTT (anti-I-A^s, I-B^s, I-J^s), B10.HTT anti-B10.S(7R) (anti-I-E^s, I-C^s, S^s, G^s), or (B10.HTT × A.TFR5)F_1 anti-A.TH (anti-I-E^s, I-C^s, S^s, G^s). The activity of the column effluents of AEF was tested at a final concentration of 25% in a secondary anti-DNP PFC response of B10.S primed spleen B cells. Both this concentration and a 50% concentration of restricted AEF yield an optimum response under the conditions used here. Fig. 2 shows that AEF activity was adsorbed by antisera reactive with determinants of either the entire I^s subregion or only the I-A^s and I-B^s subregions and not the I-E^s and I-C^s subregions. The data suggest that restricted AEF Ia antigens are determined by the *I-A* and/or *I-B* subregion. However, it should be noted that the failure of the anti-I-E^s, I-C^s, S^s, G^s sera to adsorb restricted AEF activity may be accounted for by their rather weak cytotoxic antibody activity against B10.S lymph node target cells (approximately 15% specific cytotoxicity, titer = 1:10). By contrast, about 50–60% of B10.S lymph node target cells were specifically lysed by the sera containing anti-I-A^s, I-B^s? (titer = 1:320) and anti-I-A^s, I-B^s, I-J^s (titer = 1:160) reactivity (T. L. Delovitch, unpublished observations). Accordingly, the control of restricted AEF Ia antigens by the *I-E* and *I-C* subregions may not be ruled out.

B-Cell Origin of Restricted AEF. We have previously suggested that restricted AEF Ia antigens are the products of a B cell and/or macrophage. However, the precise cellular origin of restricted AEF was not identified. Therefore, we have now analyzed the activity of a restricted AEF derived from Ia-negative responder cells and irradiated, T-cell-depleted, macrophage-depleted stimulator spleen cells. This stimulator cell population contained less

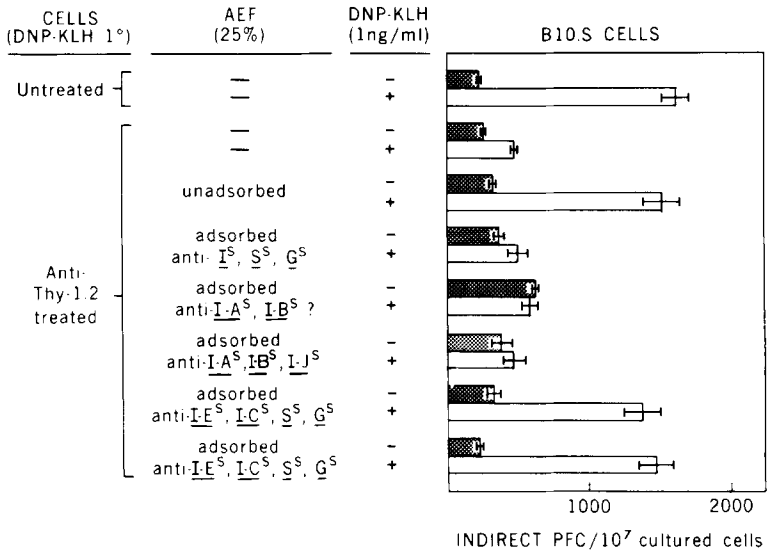

FIG. 2. Mapping of restricted AEF activity. Untreated and anti-Thy-1.2-treated DNP-KLH primed B10.S spleen cells were cultured with (+) or without (−) DNP-KLH (1 ng/ml) in the absence or presence of either unadsorbed or antibody column adsorbed restricted AEF (25% final concentration). The antisera and method used for the preparation of the immunoadsorbents are described in Materials and Methods. Indirect PFC in triplicate 6-day cultures in Click-FCS medium were enumerated by using TNP-coupled burro RBC as indicator cells. Direct PFC values ranged from 100 to 200/10^7 cultured cells and are not shown here. The results obtained from two experiments are presented as standard errors of the geometric mean.

than 1% esterase-positive cells and was thus considered to be essentially devoid of macrophages (see Materials and Methods). Fig. 3 shows that such a restricted AEF, when tested at a concentration of 50%, helped a secondary anti-DNP response of hapten-primed, macrophage-depleted T-cell-depleted spleen cells of stimulator B10.S origin but not responder B10.BR origin.

Thus, this restricted AEF displays a haplotype preference identical to that obtained by using Ia-negative responder cells and irradiated, T-cell-depleted stimulator spleen cells (12). More significantly, the presence of macrophages in the stimulator cell population does not seem to be required for the production of an active AEF. This result, taken together with the data presented in Fig. 2, suggests that restricted AEF Ia antigens are B-cell-derived products of the *I-A* and possibly *I-B* subregions.

I-Region Control of a Receptor for Restricted AEF. Genetic analysis of the activity of several factors with their appropriate target cells, derived from various *I*-region recombinant-inbred strains, has previously demonstrated a requirement for *I*-subregion identity between the factor-producing and target cell strains (4–9). In this study, we examined the ability of a restricted AEF, produced as mentioned in the previous section, to help B cells from various strains in a secondary response (Fig. 3). This AEF helped not only B10.S B cells, but also B10.HTT, B10.S(9R) and A.TH B cells. No response was obtained with A.TL B cells.

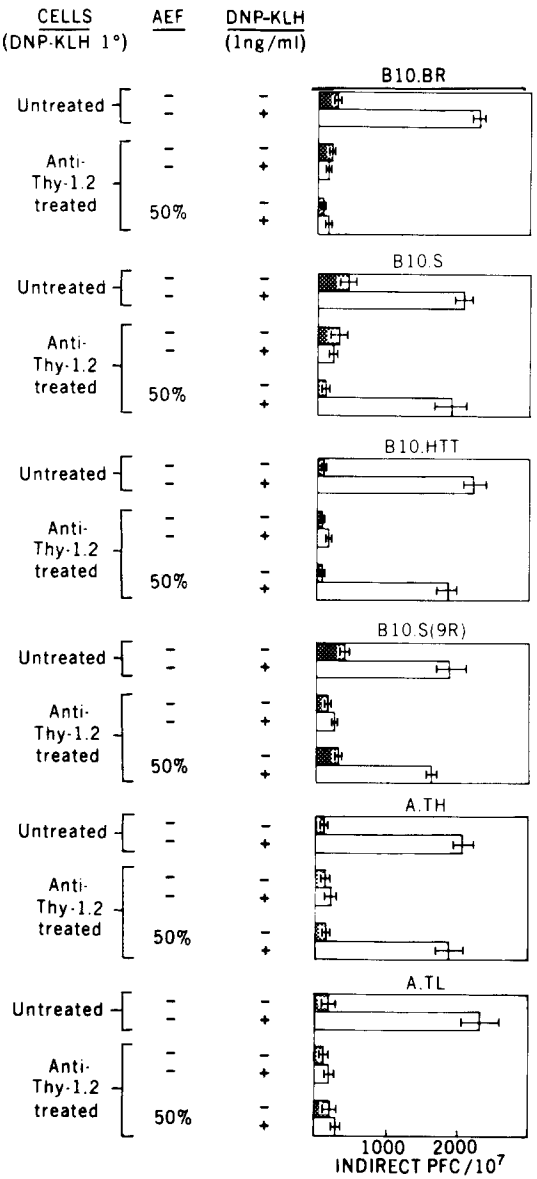

FIG. 3. Strain distribution analysis of restricted AEF activity. The activity of restricted AEF was assayed at a 50% concentration with primed, macrophage-depleted, anti-Thy-1.2-treated cells of various strains. The responses of untreated primed cells were included as controls. Culture conditions and evaluation of PFC are as in Fig. 2. The results of three experiments are shown.

Thus, restricted AEF activity is dependent upon *I*-region identity between the AEF producing strain (B10.S) and its selected target cell strain. The data also suggest that macrophage-depleted, T-cell-depleted hapten-primed spleen cells derived from B10.S, B10.HTT, B10.S(9R), and A.TH express a receptor for restricted AEF. By reference to the *H-2* haplotypes of origin of the strains tested

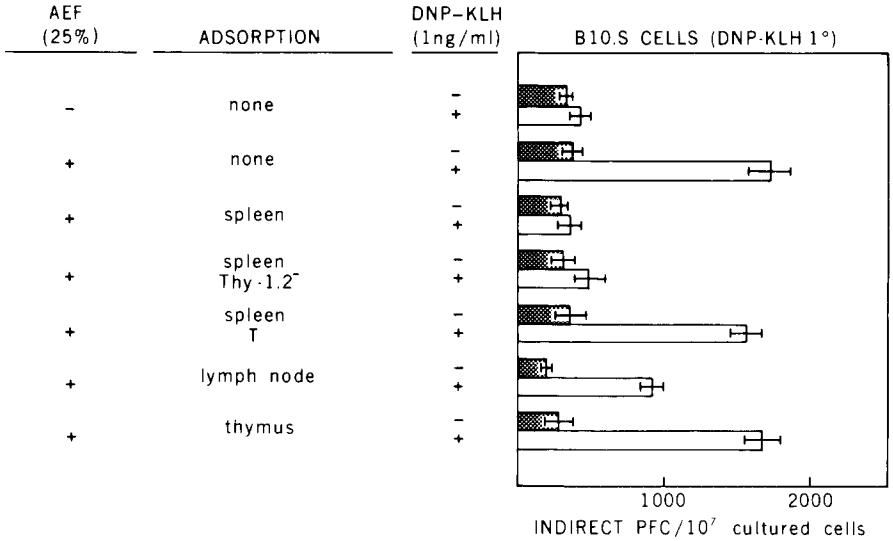

FIG. 4. Cellular adsorption of restricted AEF activity. The activity of restricted AEF was assayed at a concentration of 25% with DNP-KLH primed anti-Thy-1.2-treated B10.S spleen cells either before or after adsorption. 10^8 normal B10.S spleen cells, spleen Thy-1.2$^-$ (T-cell depleted) cells, spleen T cells (nylon wool nonadherent), lymph node cells, and thymus cells were used for adsorption. Culture conditions, number of experiments, and evaluation of PFC are as in Fig. 3.

(Table I), it is evident that this putative receptor is controlled by *H-2*-linked genes which map to the left of the *I-J* subregion. The result obtained with A.TL cells implies that the *K*-region is not involved here. It may be concluded, therefore, that the receptor for restricted AEF is a product of the *I-A* and/or *I-B* subregion.

Cellular Expression of a Receptor for Restricted AEF. The observation of cellular adsorption of the activity of a soluble lymphocyte-derived MLR suppressor factor has suggested the presence of a receptor for this factor on an activated T lymphocyte (9). The above strain distribution analysis of restricted AEF activity suggested that hapten-primed, macrophage-depleted, T-cell-depleted spleen B cells of several strains express a receptor for restricted AEF. However, no indication of whether this receptor can be found on T cells and macrophages, in addition to B cells, was presented. We therefore used the cellular adsorption technique in an attempt to identify the cell type(s) which expresses a receptor for restricted AEF.

Preliminary experiments demonstrated that restricted AEF activity was completely removed by adsorption on B10.S T-cell-depleted spleen cells obtained from either antigen-primed or normal (nonimmune) donors. Since normal donors were available in larger amount and also more frequently, all further adsorption studies were conducted with cells from normal B10.S mice. These experiments were performed with a restricted AEF prepared as described in Fig. 1. Fig. 4 shows that 10^8 spleen cells, T-cell-depleted spleen cells, and lymph node cells each adsorbed AEF activity. Complete adsorption was achieved by the spleen cell populations, while approximately only 50% adsorption was obtained with lymph node cells. These results may be explained by the relative

number of B cells in these cell populations. It should be noted that a minimum of approximately 5×10^7 B-cell-enriched T-depleted spleen cells were required for complete adsorption (T. Delovitch, J. Biggin, and F-Y. Fung, unpublished observation). By contrast, 10^8 nylon wool-purified spleen T cells (2% Ig-positive) and thymocytes showed little or no adsorbing capacity. Prior activation of the latter two cell populations with concanavalin A failed to result in their adsorption of AEF activity (data not shown). Similar data were obtained from these various cell suspensions prepared from nonimmune B10.HTT donors but not from B10.BR or B10 donors (T. Delovitch, J. Biggin, and F-Y. Fung, unpublished observations).

These studies, along with those presented in Fig. 3, indicate that restricted AEF potentiates an antibody response of B cells derived only from strains which possess a receptor for this helper factor. They also imply that B cells, and perhaps macrophages, bear this receptor. In addition, it may be argued that some residual T cells in the T-cell-depleted spleen cell population adsorbed AEF activity and therefore also bear this receptor. However, this is unlikely for two reasons. First, this cell suspension contained less than 3% Thy-1.2-positive cells, as assayed by cytotoxicity by using the same AKR/J anti-AKR/Cum anti-Thy-1.2 serum originally employed for the depletion of T cells. Second, as noted above, no adsorption was observed with 10^8 nylon wool purified spleen cells.

To further examine the cellular origin of this receptor, a comparison was made between the adsorbing capacity of B10.S T-cell-depleted spleen cells and macrophage-depleted, T-cell-depleted spleen cells. 10^8 T-cell-depleted spleen cells and 8×10^7 macrophage-depleted, T-cell-depleted spleen cells, which were recovered after removal of phagocytic cells from the original 10^8 T-cell-depleted cells in the suspension, were used. As may be seen in Fig. 5, both these cell populations adsorbed virtually all of the activity of restricted AEF. Removal of macrophages from a T-cell-depleted spleen cell suspension does not reduce the adsorption capacity of the latter cell population. Moreover, no adsorption was obtained with 9×10^7 splenic adherent cells (adherent to plastic, see reference 6 for details), while an equivalent number of splenic non-adherent cells removed all AEF activity (data not shown). Hence, it is clear that B cells, but neither macrophages nor T cells, bear a receptor for AEF. It is also evident from Fig. 5 that the adsorbing capacity of T-cell-depleted spleen cells may be eliminated by pretreatment of this cell suspension with either A.TL anti-A.TH (anti-I^s, S^s, G^s) or (B10.A × A.TL)F_1 anti-B10.S(9R) (anti-I-A^s, I-B^s?) and complement. In each instance, only about 10^7 of the original 10^8 cells in the T-cell-depleted spleen cell suspension survived treatment with these sera. These findings therefore demonstrate that those B cells which possess a receptor for restricted AEF also bear surface Ia antigens. Furthermore, since nonimmune B cells were used for these adsorption studies, B cells need not be activated by antigen to induce the expression of this receptor.

Discussion

Further evidence is provided here for a regulatory role of Ia antigens in the activation of B cells to IgG antibody production. Ia antigens determined by the stimulator haplotype have been shown to be active components of restricted AEF (12). The control of these Ia determinants has now been localized to the I-

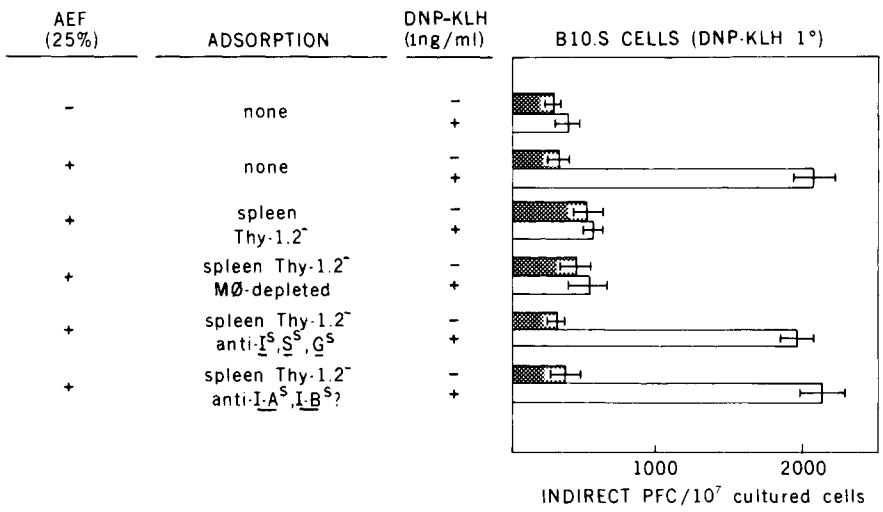

FIG. 5. Expression of a receptor for restricted AEF by Ia-positive spleen B cells. The activity of restricted AEF was assayed as in Fig. 4 at a concentration of 25% after adsorption on either 10^8 spleen Thy-1.2$^-$ (T-cell-depleted) cells, 8×10^7 macrophage (mø)-depleted spleen Thy-1.2$^-$ cells, or 10^7 spleen Thy-1.2$^-$ cells that survived lysis by either A.TL anti-A.TH(anti-I^s, S^s, G^s) or (B10.A × A.TL)F_1 anti-B10.S(9R) (anti-I-A^s, I-B^s?) plus complement (Ia-negative, T-cell-depleted cells). Culture conditions, number of experiments and evaluation of PFC are as in Fig. 3.

A and/or *I-B* subregions. It is possible that the *I-B* subregion may not be involved here. Since the *H-2* haplotype origin of the *I-B* subregion of B10.S(9R) has not yet been identified (21), it is not certain whether the (B10.A × A.TL)F_1 anti-B10.S(9R) (anti-I-A^s, I-B^s?) serum used to adsorb restricted AEF activity detects a product of the I-B^s subregion. Moreover, none of the known Ia specificities have been mapped to the I-B^s subregion (21). An antibody which reacts with only an I-A^s subregion product may therefore be sufficient to completely adsorb this activity. It should also be cautioned, however, that additional control here by the I-E^s and I-C^s subregions cannot yet be excluded. No Ia specificities have as yet been localized to these *I* subregions (21). Several investigators have therefore found it difficult to produce high-titered cytotoxic antibody reactive with products of these particular *I* subregions. Consequently, the latter findings may explain why the sera containing potential anti-I-E^s, I-C^s reactivity did not adsorb any restricted AEF activity. While these mapping data are presently somewhat inconclusive, the findings that restricted AEF is definitely a product of the *I-A* subregion is compatible with reports that several other helper factors are also determined by this subregion (4, 5, 10).

Data presented here indicate that restricted AEF may be a B-cell product. The depletion of macrophages from the stimulator cell population used to produce restricted AEF does not reduce AEF helper activity. Similarly, Eshhar et al. (22) found that the removal of adherent cells from either the responder cells or the stimulator cells does not abrogate any of the helper activity of a nonrestricted AEF. Moreover, these investigators reported that this nonrestricted AEF possesses components derived from the stimulator cell population, as well as the responder cell population. This observation confirmed our

previous findings (12). It may, therefore be concluded that macrophages do not produce AEF, nor are they required to be present for its production. These conclusions agree with those previously reported for a T cell replacing factor present in an allogeneic supernate (23). Nevertheless, it should be cautioned that the T-cell-depleted, macrophage-depleted, stimulator cell population used here to generate restricted AEF may still contain a nondetectable number of functional macrophages. In this report, macrophage depletion was assayed solely by the ability to remove esterase-positive cells. However, a small subpopulation of esterase-negative macrophages which readily phagocytose latex particles can mediate a primary IgM anti-hapten PFC response (C. Cowing, Immunology Branch, National Institutes of Health, personal communication). It is not known whether this macrophage subpopulation bears Ia antigens. Macrophages are known to be Ia positive (17), relatively radiation-resistant (6), and stimulatory in an MLR (24). Thus, it is conceivable that Ia antigens in AEF are derived either wholly or in part from esterase-negative (<1% esterase-positive), irradiated, stimulator splenic macrophages. If this is the case, a possible macrophage origin for AEF must then be considered.

MLR responder T cells have been purported to be Ia negative because pretreatment of these cells with anti-Ia serum plus complement does not inhibit their subsequent MLR response (11, 25). Other studies have shown by immunofluorescence that the Ly-1$^+$, 23$^-$ subpopulation of MLR responder T blasts, during activation across an *I*-region incompatibility, can bind Ia antigens derived from the stimulator haplotype (26). It has also been demonstrated that, during an MLR, the Ly-1$^+$, 23$^-$ subpopulation of responder T cells recognizes allogeneic Ia antigens on stimulator cells (27). Consistent with this finding is the report that the elimination of Ly-1$^+$, 23$^-$ T cells from the activated responder cells used in the generation of a nonrestricted AEF results in the loss of about 85% of AEF helper activity (22). Hence, during the in vitro production of restricted AEF, Ia antigens determined by the stimulator haplotype may be secreted by the stimulator B cell and may then bind in a specific fashion to an Ia-negative responder T cell. This responder T cell would now become Ia positive and may then release these Ia antigens, perhaps in combination with another Ia-negative component(s), into AEF. If this sequence of events were to occur, AEF Ia antigens would then have to be considered to be B-cell-derived, T-cell-dependent, products. This conclusion would support our hypothesis that AEF does indeed have a B-cell origin. It would also support our previous suggestion that Ia antigens found on T-cell membranes are passively adsorbed products synthesized by B cells (17). AEF would therefore represent the first Ia-positive helper factor to be identified as being a B-cell product. This line of reasoning would also be compatible with a previous claim that AEF is T-cell derived (28). Thus, attempts will be made in the future to determine whether Ia antigens controlled by the stimulator haplotype appear on the surface of either untreated or anti-Ia plus complement-treated MLR activated responder T cells used to produce AEF.

A strain distribution analysis of the capacity of restricted AEF to stimulate a secondary anti-DNP PFC response confirmed a previous expectation (12), that this factor can help B cells of only its own haplotype, or of haplotypes which express identical Ia antigens. An *I-A* and/or *I-B* subregion identity between the

restricted AEF producing strain and the target cell strain was required for this response. The responses obtained here are consistent with previous reports that the stimulation of an antibody response by a positive allogeneic effect involves a cooperative recognition of *I*-region products (29). By contrast, *I*-region products are not recognized during a negative allogeneic effect which results from the suppression of an immune response by allogeneic T cells (27, 29).

Thus, the *I*-subregion determinants which are present in restricted AEF must also appear on the surface of its target cell. This suggests that the target cell bears a specific receptor for restricted AEF and that this receptor and restricted AEF are both products of the same *I*-subregion(s). Since it has been tentatively concluded above that restricted AEF Ia molecules are determined by *I-A*, this *I*-subregion may also control the receptor postulated to exist here. The suggestion that AEF and its receptor are encoded by the *I-A* subregion would agree closely with the previously reported control by this *I*-subregion of other helper factors and their receptors (4–6, 10).

Studies performed here demonstrate that the B cell represents the target cell of AEF. Furthermore, they suggest that an Ia-positive B cell expresses a receptor for AEF. It appears that neither a prior antigen-activation of the B cell nor a specific surface modulation event is necessary for the expression and function of this receptor. These data therefore strongly support the earlier evidence that AEF produced in the usual manner (12, 28) and other antigen-nonspecific T-cell replacing factors (30, 31) act directly on B cells during either a primary IgM or a secondary IgG antibody response. The latter factors described both stimulate a B-cell response which is macrophage independent.

T cells and macrophages from either unprimed or antigen-primed donors apparently do not express a receptor for restricted AEF, or otherwise possess a substantially lower density of receptors which is below the limit of sensitivity of detection achieved here. These results differ from those previously obtained with Ia-positive T-cell-derived suppressor factors and an Ia-positive macrophage-derived helper factor, which are purported to possess *I*-region determined receptors on antigen-activated T cells (7, 9) and normal T cells (6), respectively.

As mentioned above, several in vitro studies of IgG antibody synthesis have shown a requirement for *I*-region compatibility between several helper factors and their respective receptors. Similarly, *I*-region identity is required for effective T-cell B-cell interaction in vivo. Katz et al. (32) have reported that antigen-primed T and B cells must share an *I-A* subregion identity to cooperate for a secondary IgG antibody response. Press et al. (33) have demonstrated that a secondary IgG anti-DNP response may be mediated by a specific recognition by carrier-primed T cells of syngeneic Ia antigens on hapten-primed B cells. The same conclusion was reached by Pierce and Klinman (34) who showed that the transfer of primary DNP-specific B cells into allogeneic carrier-primed recipients resulted in only an IgM anti-DNP response; the transfer of either primary or secondary DNP-specific B cells into syngeneic carrier-primed recipients resulted in only an IgG response (35). In the latter studies, stimulation of IgG production was dependent on an identity in the *I-A* subregion between the collaborating T and B cells.

The apparent need for *I*-region compatibility exemplified in vitro in this study may therefore represent an event of normal B-cell activation during IgG

antibody synthesis in vivo. It may be envisaged, as proposed above, that after antigenic stimulation a T cell recognizes Ia determinants on a B cell and then passively acquires these Ia molecules onto its surface. This Ia-positive T cell may then interact with a syngeneic antigen-presenting cell, i.e., a macrophage. Such an interaction might induce the release from the T cell of the immunizing antigen or fragment thereof, Ia antigens, and perhaps other membrane-associated components. These T-cell released components, which may or may not become associated in a complex structure, might be recognized and bind to their specific receptors on a B cell. Such a mechanism would allow for an *I*-region identity to be achieved at both the macrophage T cell and T cell B cell levels of interaction. It would also enable a T cell to recruit and collaborate with an Ia compatible B cell.

A two-signal hypothesis has been proposed for B-cell triggering (36, 37). Antigen represents the first signal that binds to its B-cell immunoglobulin receptor. A T-cell factor or thymus-independent antigen may act as a second signal and bind to another B-cell surface receptor. AEF, which consists of Ia antigens and perhaps additional Ia-negative components, may mediate T cell B cell collaboration by serving as a second signal given to the B cell during the stimulation of an antibody response. Hence, a specific recognition between an Ia molecule in AEF and a complementary component(s) in its B-cell receptor could conceivably occur during B-cell activation.

The immunochemical nature of the receptor for AEF is not known. It has been suggested here that this receptor is an *I*-region gene product. The possibility has also been raised that the receptor for another nonantigen-specific mediator of B-cell responses bears Ia determinants (38). However, it is also possible that these receptors are not comprised of Ia antigens. One alternative to be considered in the present study is that Ia antigens on the B cell may become associated with the receptor for AEF only after the formation of a factor receptor complex. The receptor may in fact be controlled by a non-*H-2*-linked gene. It may recognize an Ia-negative T-cell-derived component of AEF which is complexed to an Ia molecule. The *I*-region compatibility requirement discussed earlier may be fulfilled solely by the interaction of AEF Ia antigens and syngeneic Ia antigens on a nonreceptor portion of the B-cell membrane. These possibilities may only be analysed by a comparative biochemical analysis of this B-cell receptor (if it can be isolated), B-cell surface Ia antigens and Ia antigens in AEF. Such studies are currently in progress.

Summary

A genetically restricted allogeneic effect factor (AEF) derived from a mixed lymphocyte culture reaction between Ia-negative activated responder cells and irradiated T-cell-depleted stimulator cells was characterized. Restricted AEF is a B-cell-derived soluble helper factor which consists in part of Ia antigens controlled by the *I-A* subregion of the stimulator haplotype; additional control by the *I-B*, *I-E*, and *I-C* subregions, although unlikely, could not be excluded. This factor helps B cells of only its own haplotype or of haplotypes which carry an *I-A* and/or *I-B* subregion identity. Unprimed as well as hapten-primed Ia-positive B cells express a receptor for restricted AEF. The results indicate that the B-cell receptor for AEF is determined by the *I-A* subregion. Both restricted

AEF and its receptor may therefore be products of the same *I*-region gene(s). The data are compatible with the hypothesis that the AEF Ia antigens serve as a second signal required for B-cell activation to IgG antibody production.

We thank Doctors A. Cunningham, E. Gelfand, and A. Marks for their helpful suggestions in the preparation of this manuscript. We also thank Ms. Patricia Camden for her fine secretarial assistance.

Received for publication 20 October 1977.

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