# IN VITRO ANALYSIS OF ALLOGENEIC LYMPHOCYTE INTERACTION

## V. Identification and Characterization of Two Components of Allogeneic Effect Factor, One of Which Displays *H-2*-restricted Helper Activity and the Other, T Cell-Growth Factor Activity*

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During differentiation in a thymus, T lymphocytes learn to recognize self alloantigens determined by the H-2 major histocompatibility complex. This imposes certain genetic restrictions on lymphocyte interaction during T cell-mediated immune responses (1–3). For example, antigen-specific helper T cells acquire a binding capacity not only for a given foreign antigen but also for Ia alloantigens of the host haplotype. Our previous studies have shown that soluble allogeneic effect factors (AEF)[1] derived from mixed lymphocyte reaction (MLR) cultures of graft-versus-host reaction (GVHR)-activated responder T cells and either *H-2*- (4, 5), *I*-region- (6), *I*-subregion- (7, 8) or *Mls*-locus- (9) incompatible irradiated stimulator cells are genetically restricted in their helper activities. They each preferentially help primary and secondary antibody responses of B cells of the stimulator haplotype. The activity of several of these factors may be absorbed by alloantibodies reactive with *I-A*- (5, 6) or *I-J*- (8) subregion products of the stimulator haplotype but not of the responder haplotype. The genetic restriction of these responses may be controlled in part by the acquisition by GVHR donor and MLR responder cells of GVHR host and MLR stimulator Ia antigens (10). Thus, although it is apparent that both helper T cells and AEF helper

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[1] *Abbreviations used in this paper:* AEF, allogeneic effect factors; BSA, bovine serum albumin; C′, rabbit serum complement; Con A, concanavalin A; CRI, cross-reactive idiotype; CTL, cytotoxic T lymphocytes; CTLL, CTL harvested from long-term T cell-growth factor-dependent cultures; 1-D, 2-D, one and two dimensional; GVHR, graft versus host reaction; IEF, isoelectric focusing; MLR, mixed lymphocyte reaction; PBS, phosphate-buffered saline; PFC, plaque-forming cells; pI, isoelectric point(s); SaCI, *Staphylococcus aureus*, Cowan I strain; SDS, sodium dodecyl sulphate; SDS-PAGE, SDS-polyacrylamide slab gel electrophoresis; SRBC, BRBC, sheep and burro erythrocytes; TCGF, T cell-growth factor; TRF, T-cell-replacing factor.

factors recognize an immunogen in association with syngeneic Ia antigens, the molecular basis for this associative recognition is unknown. It was therefore of interest to biochemically and biologically characterize the component(s) of an AEF that is *H-2* restricted in its activity for a B cell response of the stimulator haplotype.

In this report, we demonstrate that a genetically restricted AEF produced across an *H-2* incompatibility consists of two main active components. One component, of ~30,000–35,000 mol wt, possesses identical biochemical and biological properties to murine T cell-growth factor (TCGF) (11) and is not genetically restricted in its activity. The other component has a mol wt of ~68,000, differs both biochemically and biologically from TCGF, and is genetically restricted in its helper activity for B cells of the stimulator haplotype and of haplotypes that share *I-A*-region identity with the stimulator haplotype. It is suggested that the latter AEF component is an alloactivated responder T cell-derived receptor for stimulator cell *I-A*-controlled Ia alloantigens.

## Materials and Methods

*Mice.* Mice used in this study were either bred and maintained at the University of Toronto, Toronto, Canada or purchased from The Jackson Laboratory, Bar Harbor, Maine.

*Antigens.* Burro and sheep erythrocytes were purchased from Colorado Serum Co., Denver, Colo.

*Antisera.* The anti-*H-2* and anti-Ia alloantisera and the rabbit anti-bovine serum albumin (BSA) serum used were produced at the University of Toronto. A303 is a monoclonal antibody that detects Ia antigens whose $\alpha$-chain subunit ($E_\alpha$) is controlled by a gene in $I\text{-}E^k$ and whose $\beta$-chain subunit ($A_e$) by a gene in $I\text{-}A^k$ (12). Either a (B6.PL-Thy-1.1 × PL/J)$F_1$ anti-C57BL/6 congenic anti-Thy-1.2 serum or an anti-Thy-1.2 hybridoma antibody (13) was used to deplete suspensions of T cells. Rabbit anti-mouse $F_v$ and chicken anti-mouse Ig were kindly provided by Dr. M. Feldmann, Department of Zoology, University College, London, England. Rabbit anti-mouse $V_H$, rabbit anti-mouse $V_L$, and normal rabbit Ig were generously supplied by Dr. M. Feldmann but were produced by Dr. D. Givol, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel. A goat anti-Scripps virus gp 70 antiserum was kindly provided by Dr. S. Kennel, Oak Ridge National Laboratory, Oak Ridge, Tenn. A rabbit antiserum against the cross-reactive idiotype (CRI) of serum anti-*p*-azophenylarsonate antibodies produced in A/J mice (14) was kindly supplied by Dr. J. D. Capra, Department of Microbiology, Southwestern University of Texas, Dallas, Tex.

*Preparation of AEF.* AEF was produced across an *H-2* incompatibility by using GVHR-activated A.SW ($H\text{-}2^s$) responder T cells and irradiated A/WySn ($H\text{-}2^a$) stimulator spleen cells, according to procedures previously described (4–9). Briefly, spleen cells were recovered from irradiated (800 rad) A/WySn recipients that were injected intravenously 5 d earlier with $10^8$ A.SW thymocytes. Supernates were recovered from 16- to 20-h MLR cultures (1-ml volume) in a serum-free RPMI-1640 medium (8) of $10^7$ A.SW activated responder spleen T cells and $10^7$ irradiated (3,000 rad) normal stimulator A/WySn spleen lymphocytes. They were then assayed for AEF activity.

*Chromatographic Resolution of AEF.* All chromatography was performed at 4°C using sterile buffers, essentially as described before (11).

Approximately 15 ml of unseparated AEF was applied to a 2- × 90-cm column of Ultrogel ACA 54 (Fisher Scientific Co., Pittsburgh, Pa.) previously equilibrated with 0.9% NaCl. Column fractions (8 ml) were eluted in this buffer and their $OD_{280}$ were continuously monitored with an LKB Uvicord II (LKB Instruments, Inc., Rockville, Md.). The column was calibrated with the following molecular weight standards: human serum albumin (mol wt 67,000), ovalbumin (mol wt 43,000), TCGF (mol wt 30,000), and cytochrome *c* (mol wt 12,500).

Biologically active fractions (see Fig. 2A, pools I and II) from the ACA 54 column were dialyzed against 0.04 M NaCl buffered in 0.01 M Hepes, pH 7.2, and applied to a 1.5- × 8-cm column of DEAE-Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden) previously equili-

brated in this buffer. Column fractions (8 ml) were eluted using a 120-ml linear gradient of 0.04–0.5 M NaCl buffered in 0.01 M Hepes, pH 7.2, and their ionic strengths monitored with a conductivity meter.

Active fractions that eluted between 0.05 and 0.1 M NaCl (Fig. 3, pool III) from the DEAE-Sephacel column were dialyzed against 1% glycine, adjusted to contain 2% Ampholines (pH 3–10; LKB Instruments, Inc., Rockville, Md) and then mixed with 5 g of Ultrodex (treated Sephadex G-75; LKB Instruments, Inc.). The gel suspension was spread on a horizontal flat-bed isoelectric focusing (IEF) tray and electrophoresis was carried out at 5°C for 26–30 h using a constant current of 8 mA (see Fig. 4A) (11). After IEF, gel sections were made, analyzed for their pH, and then transferred to 1- × 5-cm columns that were eluted with 5–10 ml sterile water. Each sample was dialyzed against 0.04 M NaCl to remove Ampholines, lyophilized, and finally resuspended in 200 μl sterile water before assay.

Alternatively, selected DEAE column fractions were further resolved by IEF on a 110-ml preparative column (model 8100-1; LKB Instruments, Inc.) that was stabilized with a gradient of 0–60% glycerol (15). Ampholytes (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc.) in the pH range of 3–10 were added to the gradient at a 1:40 dilution. Electrophoresis was carried out for 25 h at 4°C. 40 2.5-ml fractions were collected, their pH determined, and then neutralized to pH 7.2 using 1 M Tris base before assay.

*Radioiodination of AEF.* Biologically active AEF fractions separated by IEF were dialyzed using sterilized Spectrapor membranes (3,500-mol wt cutoff; Spectrapor Medical Industries, Los Angeles, Calif.) for 48 h against phosphate-buffered saline (PBS), pH 7.4, with several buffer changes. They were then concentrated with Millipore (Millipore Corp., Bedford, Mass.) immersible CX ultrafiltration units (10,000-mol wt cutoff), and adjusted to pH 8.5 using 1 M PBS, pH 9. Samples were labeled with $^{125}$I using the Bolten-Hunter reagent (>1375 Ci/mmole sp act; Amersham Corp., Oakville, Ontario) according to the method of Langone et al. (16), with the exception that a PBS buffer that contained 0.1% gelatin and 10 mM Tris-HCl, pH 7.4, was used (12).

*Gel Electrophoresis of AEF.* $^{125}$I-labeled AEF fractions were analyzed under reducing and nonreducing conditions by one-dimensional (1-D) sodium dodecyl sulfate (SDS)-polyacrylamide (10% wt:vol) slab gel electrophoresis (SDS-PAGE) or were subjected to electrophoresis on a two-dimensional (2-D) gel according to the method of O'Farrell (17), as previously described (18). Dried gels were impregnated with EN$^3$HANCE (New England Nuclear, Boston Mass.) and were then exposed to preflashed (19) Kodak XR-1 film (Eastman Kodak Co., Rochester, N. Y.) using a Cronex Lightning Plus image-intensifying screen (Du Pont Canada Inc., Markham, Ontario, Canada).

*Immunoprecipitation of of AEF.* Aliquots (50 μl) of $^{125}$I-labeled AEF previously purified by IEF and containing ~5,000–6,000 cpm were reacted with 25 μl of various antisera for 18 h at 4°C. Samples were further treated for 30 min at 4°C with 100 μl of a 10% (vol:vol) heat-killed, formalin-fixed suspension of *Staphylococcus aureus*, Cowan I strain (SaCI). They were then centrifuged at 6,300 *g* for 15 min through 1 ml of 2 M sucrose in 0.02 M Tris-HCl, pH 7.4, to remove unbound antibody and to dissociate nonspecifically formed immune complexes. After washing of the pellets three times with 1 ml of PBS that contained 0.5% Nonidet P-40 and 5 mM KI, they were resuspended at 23°C in 50 μl of a solution of 2% (vol:vol) 2-mercaptoethanol and 5% (vol:vol) SDS, centrifuged, and the radioactivity in the supernates was determined in a Beckman 300 gamma counter (Beckman Instruments, Inc., Fullerton, Calif.).

*Biological Assays of AEF.* AEF fractions separated by ACA 54, DEAE-Sephacel and IEF were assayed for their capacity to promote (*a*) the proliferation of mouse cytotoxic T lymphocytes (CTC) harvested from long-term TCGF-dependent cultures (CTLL cells), (*b*) concanavalin A (Con A)-induced mitogenesis of normal mouse thymocytes, (*c*) cytolytic reactivity to alloantigens in mouse thymocyte and nude spleen cell cultures, and (*d*) antibody responses to either sheep or burro erythrocytes (SRBC, BRBC) in cultures of either nude mouse spleen or T cell-depleted normal spleen cells. These assays were performed as previously reported (8, 9, 11). After each biochemical method of resolution of AEF, aliquots of isolated fractions were examined for activity in each of the above biological tests. Fractions were assayed in replicate at concentrations ranging between 5 and 50% (vol:vol). Peak responses in thymocyte mitogenesis, CTL, and antibody production assays were usually observed at a sample concentration of ~5–10%.

## Results

*H-2-restricted Helper Activity of Unfractionated AEF.* Table I summarizes our previous studies in which 9 out of 10 AEF generated across either *H-2* ([4, 5]; and T. Delovitch. Unpublished observations.), *I*-region (6), *I*-subregion (7, 8), or *Mls*-locus (9) incompatibility displayed an *H-2* restriction in their helper activity for B cells of the stimulator haplotype. An AEF (AEF-1) generated using B10.BR activated responder T cells and B10.S irradiated stimulator spleen cells was the only AEF that elicited an equivalent response of B cells of the responder and stimulator haplotypes (4). However, it showed an apparent genetic restriction as it helped B cells of only some of the haplotypes tested. The difference in activity of this AEF when compared with that of the nine others listed in Table I may be because: (*a*) it was produced in the absence of the antiserum plus complement (C′) treatments of the responder and stimulator cells used for AEF-2 (5) and (*b*) it was produced using an x-ray source and tissue culture medium that differed from those used to generate AEF 3–10 (4–9). The conditions used for the latter AEF were those indicated for AEF-4 in the Materials and Methods section of this report. Because genetic restriction of helper activity was displayed for all AEF examined and in 90% of the cases AEF helper activity was restricted to cells of the stimulator haplotype, it was conceivable that a major component, which mediated this type of *H-2* restriction, was present in each of these AEF.

Thus, a biochemical and biological analysis of an AEF produced across an *H-2* incompatibility was performed. Because an AEF generated across an *H-2* difference yields the strongest helper activity (Table I) and the $H\text{-}2^s$ and $H\text{-}2^k$ haplotypes differ most widely in their expression of Ia antigenic specificities, AEF was derived from MLR cultures of A.SW ($H\text{-}2^s$) GVHR-activated responder T cells and A/WySn ($H\text{-}2^a$) irradiated stimulator spleen cells. These strains were in greater supply in our

TABLE I

*Helper Activities of Various AEF*

| Strain* | | Incompatibility‡ | Activity§ | | H-2 restriction |
|---|---|---|---|---|---|
| Responder | Stimulator | | Responder | Stimulator | |
| 1. B10.BR | B10.S | $H\text{-}2^s$ | ++++ | ++++ | No |
| 2. B10.BR ($Ia^-$) | B10.S (Thy-1.2$^-$) | $H\text{-}2^s$ | ± | ++++ | Yes |
| 3. B10.S | B10.BR | $H\text{-}2^k$ | ± | ++++ | Yes |
| 4. A.SW | A/WySn | $H\text{-}2^k$ | + | ++++ | Yes |
| 5. A.TH | A.TL | $I^k$,*TL?*,*Qa?* | + | ++++ | Yes |
| 6. (A.TH × B10.HTT)$F_1$ | A.TL | $I\text{-}A^k$,$I\text{-}B^k$,$I\text{-}J^k$ | ± | +++ | Yes |
| 7. B10.A(3R) | B10.A(5R) | $I\text{-}J^k$ | − | ++ | Yes |
| 8. B10.A(5R) | B10.A(3R) | $I\text{-}J^b$ | − | ++ | Yes |
| 9. B10.S(7R) | B10.HTT | $I\text{-}EC^k$,*TL?*,*Qa?* | ± | +++ | Yes |
| 10. C3H/HeJ | B10.BR | $Mls^b$ | − | ++ | Yes |

* Strains used for the production of AEF.

‡ Genetic incompatibility in the GVHR and MLR phases of AEF production.

§ Helper activity with T-cell depleted spleen cells of either responder or stimulator haplotype. Relative strengths of AEF helper activity are shown as: ++++ (2,000–3,000 PFC/$10^7$ cells), +++ (1,000–2,000 PFC/$10^7$ cells), ++ (300–1,000 PFC/$10^7$ cells), + (100–300 PFC/$10^7$ cells), ± (50–100 PFC/$10^7$ cells), and − (<50 PFC/$10^7$ cells).

colony than the histocompatible B10.S($H\text{-}2^s$) and B10.BR($H\text{-}2^k$) strains previously used for AEF 1–3. They also enabled the use of several recombinants of the $H\text{-}2^s$ and $H\text{-}2^k$ haplotypes to carry out genetic mapping studies of AEF helper activity.

Fig. 1 A demonstrates that AEF preferentially helps A/WySn, but not A.SW, T cell-depleted spleen cells. Optimum helper activity was obtained at a concentration of 0.01% (i.e., a dilution of 1:10,000). AEF helper activity is, therefore, genetically restricted for B cells of the stimulator haplotype. This AEF also helps B10.A, B10.BR, B10.S(8R), B10.A(4R), A.TL, and BALB/c B cells; but not A.TH, B10.S, B10, B10.A(5R), and B10.HTT B cells (data not shown). Thus, AEF seems to help primarily B cells of haplotypes that share $I\text{-}A^k$-subregion identity with the stimulator haplotype. The only exception to this *I-A* identity requirement for AEF activity is the BALB/c strain; the reason that AEF helps this strain is presently not understood and

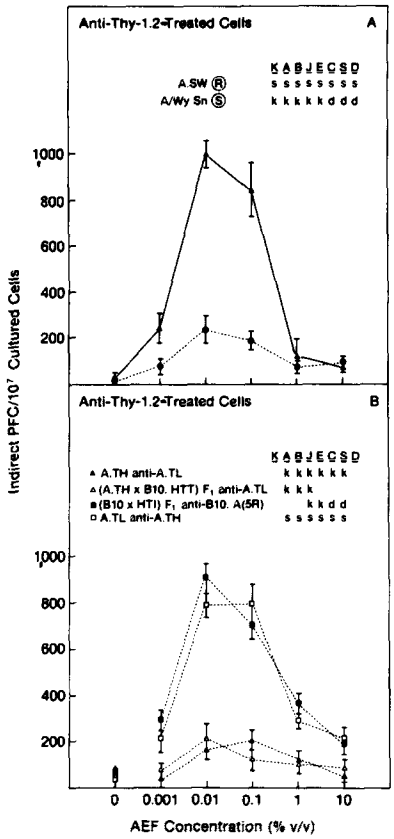


FIG. 1. *H-2* restriction and immunoadsorption of AEF helper activity. (A) Anti-Thy-1.2 plus C′-treated spleen cells ($10^6$) from either responder (R) A.SW (●) or stimulator (S) A/WySn (▲) unprimed mice were cultured with or without BRBC ($3 \times 10^6$) in the absence or presence of AEF at various final concentrations ranging from 0.0001 to 10% (vol:vol). Direct PFC in triplicate 5-d cultures in RPMI-1640 medium containing 5% fetal calf serum were enumerated and are presented as SEM (arithmetic). The results of two experiments are shown. Background PFC values obtained in the absence of antigen ranged from 0 to 100 and have been subtracted. The haplotype origin of the *H-2* regions of the strains used are indicated. (B) Anti-Thy-1.2 plus C′-treated A/WySn spleen cells were cultured with or without antibody-column adsorbed AEF. The anti-Ia sera used for adsorption and their potential reactivities are presented. Other conditions used are as in (A) above.

may be related to a structural homology of $Ia^k$ and $Ia^d$ antigens (see Discussion). Compatible with this mapping data is the finding that AEF helper activity for A/WySn T cell-depleted spleen cells could be removed by immunoadsorption on cyanogen bromide-activated Sepharose 4B columns conjugated with either A.TH anti-A.TL (anti-$I^k$,$S^k$) or (A.TH × B 10.HTT)$F_1$ anti-A.TL (anti-$I\text{-}A^k$,$I\text{-}B^k$,$I\text{-}J^k$), but not with (B10 × HTI)$F_1$ anti-B10.A(5R) (anti-$I\text{-}J^k$,$I\text{-}E^k$,$I\text{-}C^y$,$S^d$) or A.TL anti-A.TH (anti-$I^s$,$S^s$) (Fig. 1B). These observations suggested that unfractionated AEF possesses a component(s), potentially responder T cell membrane-derived, that can recognize and bind to Ia antigens determined by the $I\text{-}A^k$ subregion of the stimulator haplotype. To attempt to understand the molecular basis of T cell recognition of Ia alloantigens, it was considered important to identify such an AEF component. A biochemical characterization of AEF was, therefore, performed. AEF was subjected to various methods of resolution and the fractions obtained examined for their activity in several biological assays.

*Gel Filtration.* Approximately 15 ml of AEF harvested in a serum-free RPMI-1640 medium supplemented with the protease inhibitor Trasylol (8) was fractionated according to size by chromatography in 0.9% NaCl on an ACA 54 column. The fractions obtained were assayed at a 1:10 dilution for their ability to stimulate a primary in vitro anti-SRBC direct plaque-forming cell (PFC) response of either nude spleen cells or T cell-depleted spleen cells under culture conditions that were limiting for helper T cells (11). Fig. 2A shows that fractions eluting in the 50,000- to 70,000- and 25,000- to 35,000-mol wt ranges, designated pools I and II, respectively, potentiate a PFC response of BALB/c ($H\text{-}2^d$) nude spleen cells. The helper activity, expressed as the fraction of responding microcultures, of pool I was about twofold greater than that of pool II. This AEF helper activity for BALB/c nude spleen cells is consistent with the observation of AEF helper activity for BALB/c T cell-depleted spleen cells mentioned above. Pool II displays TCGF-like activity when tested (fractions were diluted 1:5) for their capacity to stimulate the growth of TCGF-dependent CTLL cells (Fig. 2A), and promotes Con A-induced mitogenesis of thymocytes and the generation of cytotoxic T cells in either thymocyte or nude spleen cell cultures (J. Watson. Unpublished observations.). By contrast, pool I does not possess any of the latter three biological activities. Pool I also differs from pool II because it helps a PFC response of B10.A but not B10.S T cell-depleted spleen cells (Fig. 2B). Again, the helper activity of pool I was about twice that of pool II. This indicates that the helper activity of pool I is restricted to B cells that are histocompatible with the stimulator haplotype and not the responder haplotype; the helper activity of pool II is not genetically restricted. Thus, gel filtration demonstrates that AEF may be resolved into two main components, pool I and pool II, which differ in their molecular size and biological activity.

*Ion-Exchange Chromatography.* To further resolve any differences in the biochemical properties of pools I and II eluted from ACA 54, these biologically active components were analyzed by ion-exchange chromatography. The fractions collected were analyzed at a 1:10 dilution for their helper activity with BALB/c nude spleen cells and for their TCGF-like activity at a 1:5 dilution with CTLL cells.

It is evident from Fig. 3A that pool I eluted from DEAE-Sephacel with a major peak between 0.05 M and 0.1 M NaCl-Hepes, pH 7.2, and a minor peak between 0.15 M and 0.2 M NaCl-Hepes, pH 7.2. A similar elution profile was obtained for

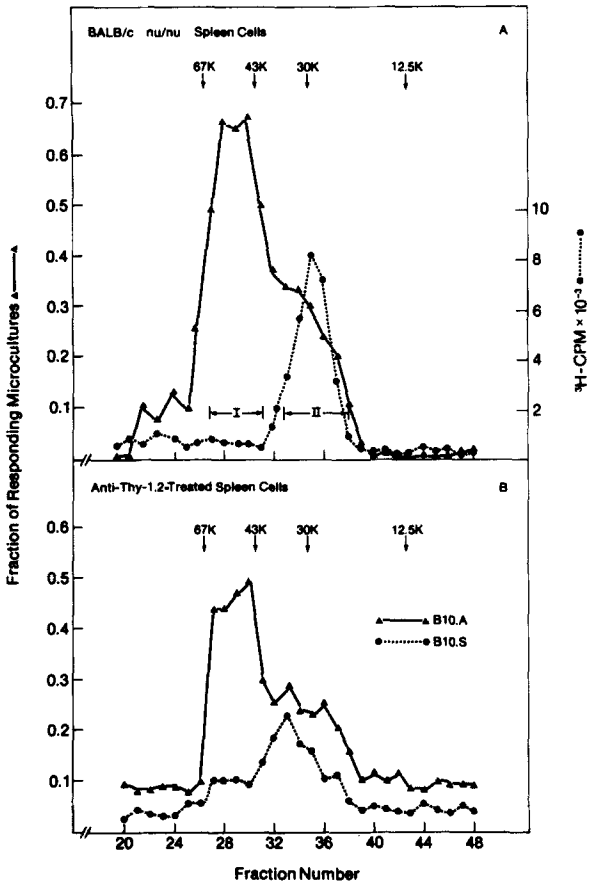


FIG. 2. Assay of AEF activity after gel filtration on ACA 54. (A) Column fractions were assayed for their capacity to promote at a 1:10 dilution a primary in vitro anti-SRBC direct PFC response of BALB/c nude spleen cells (▲) or to stimulate at a 1:5 dilution CTLL cell growth (●). PFC results are expressed in terms of the fraction of responding microcultures in the 120 cultures tested for each column fraction (11). Results of cell growth are expressed as [$^3$H]thymidine counts per minute incorporated into trichloroacetic acid-precipitable DNA. The molecular weight markers used to calibrate the column were human serum albumin (67,000), ovalbumin (43,000), TCGF (~30,000), and cytochrome *c* (12,500). (B) Column fractions were assayed at a 1:10 dilution for their ability to stimulate an anti-SRBC response of either B10.S (●) or B10.A (▲) T cell-depleted spleen cells. In (A) and (B) above, two distinct components, pools I and II, were identified.

pool II (Fig. 3B). The contamination of pool I with pool II in Fig. 3A and vice-versa in Fig. 3B presumably arises from the manner in which pools I and II were originally selected (Fig. 2). Interestingly, AEF helper activity resided in fractions eluting both at low (0.05–0.1 M) and high (0.15–0.2 M) NaCl concentrations, whereas TCGF-like activity was present only in those fractions eluted at the higher NaCl concentration (Fig. 3B). Thus pool I activity may be fractionated between 0.05 and 0.1 M NaCl and pool II activity may be fractionated between 0.15 and 0.2 M NaCl. This further demonstrates that the biologically active pools I and II consist of biochemically different molecules. Because pool I lacks TCGF-like activity but possesses *H-2*-restricted helper activity, it was of interest to further characterize the major peak eluted from DEAE-Sephacel at 0.05–0.1 M NaCl, which was designated as pool III.

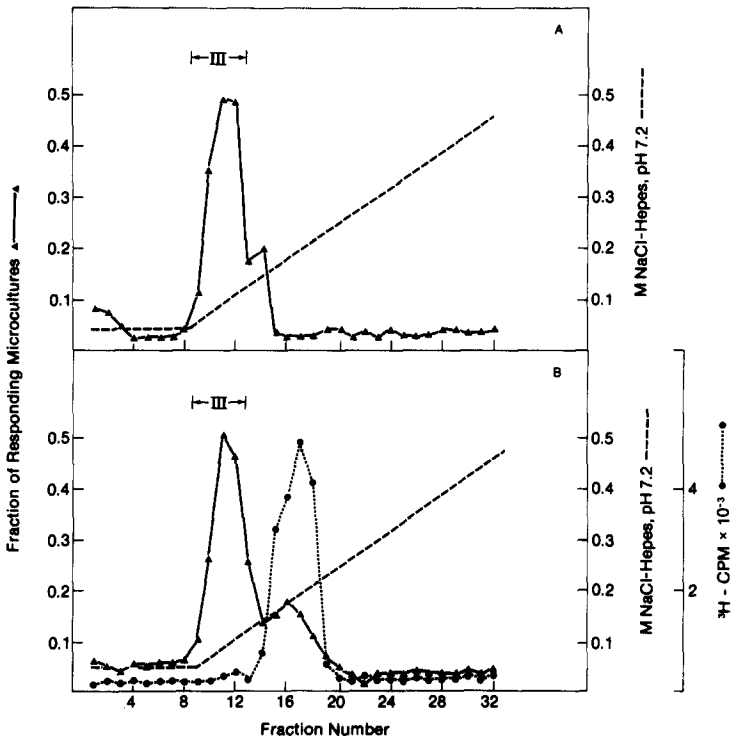


FIG. 3. Assay of AEF activity after ion-exchange chromatography on DEAE-Sephacel. Active AEF components obtained from ACA 54 pool I (A) and ACA 54 pool II (B) were fractionated on DEAE-Sephacel and assayed either for their helper activity with BALB/c nude spleen cells (▲) or their ability to stimulate CTLL cell growth (●). The concentration gradient of 0.04–0.4 M NaCl-Hepes, pH 7.2, is indicated (---). Fractions eluted between 0.05 and 0.1 M NaCl were designated as pool III.

*IEF.* AEF pool III collected from the DEAE-Sephacel eluates shown in Fig. 3 was separated by IEF using a pH gradient of 3–10. Two major peaks of helper activity (assayed at a 1:3 dilution) for B10.A T cell-depleted spleen cells were obtained by IEF on a horizontal flat-bed of Sephadex G-75 (Fig. 4A). The isoelectric point (pI) of peak I was 5.8 and of peak II was 6.2. It should be noted that a broad peak of activity with a pI in the 5.5–6.5 range was sometimes observed (J. Watson. Unpublished observation.). It, therefore, remains uncertain whether peaks I and II represent two distinct molecules of similar molecular size and different molecular charge.

When pool III of the DEAE-Sephacel column was separated by IEF on a preparative vertical column stabilized with a gradient of 0–60% glycerol, one predominant peak with a pI in the range of 5.5–6.0 was observed (Fig. 4B). The latter peak preferentially helped an anti-SRBC direct PFC response of T cell-depleted spleen cells of the A/WySn stimulator haplotype and not of the A.SW responder haplotype. Thus, the *H-2*-restricted helper activity of AEF was maintained throughout the several biochemical methods of resolution used and seems to reside with a major component, component I, of pI 5.5–6.0.

*Gel Electrophoresis.* To further analyze whether the *H-2*-restricted AEF helper

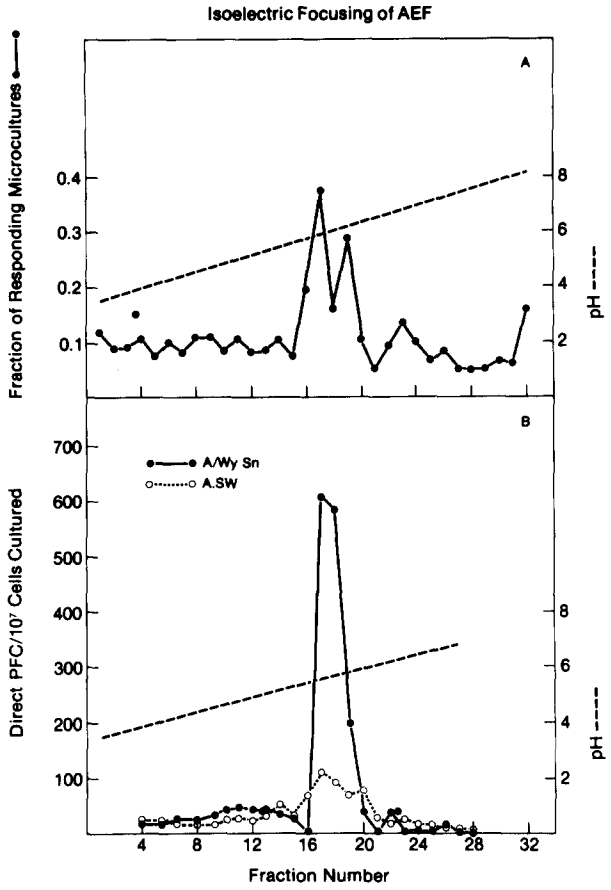


FIG. 4. Assay of AEF activity after IEF (pH 3–10). Pool III from DEAE-Sephacel was analyzed by either horizontal flat-bed IEF (A) or preparative vertical column IEF (B). Fractions obtained were diluted 1:3 and assayed for their helper activity with either B10.A T cell-depleted spleen cells (A) or A.SW (●) and A/WySn (○) T cell-depleted spleen cells (B).

activity of pI 5.5–6.0 is attributable to one or perhaps more than one component, the IEF fractions containing helper activity were characterized by gel electrophoresis.

Fractions 17 and 19 of AEF component I shown in Fig. 4A were each dialyzed to remove Ampholines, concentrated, and labeled with $^{125}I$ using the Bolton-Hunter reagent. They were then fractionated on Sephadex G-25 to remove free $^{125}I$, dialyzed, concentrated, and applied to a lentil lectin affinity column; the unbound and bound fractions were lyophilized, dissolved, and analyzed under reducing conditions by 1-D 10% SDS-PAGE. The gel fluorogram presented in Fig. 5 demonstrates that the lentil lectin-unbound material of fractions 17 (track A) and 19 (track B) consists of a major band in the 68,000-mol wt (Fig. 5, 68 K) region. This band is absent from the lentil lectin-bound material of these fractions (Fig. 5, tracks C and D). Another band at ~10,000–15,000 mol wt which comigrated with the Coomassie blue dye front was present in both of the unbound and bound fractions (Fig. 5, tracks A–D). These data suggest that the 68,000-mol wt component does not possess mannose residues and, therefore, may not be a glycoprotein. The glycoprotein nature of the 10,000- to 15,000-

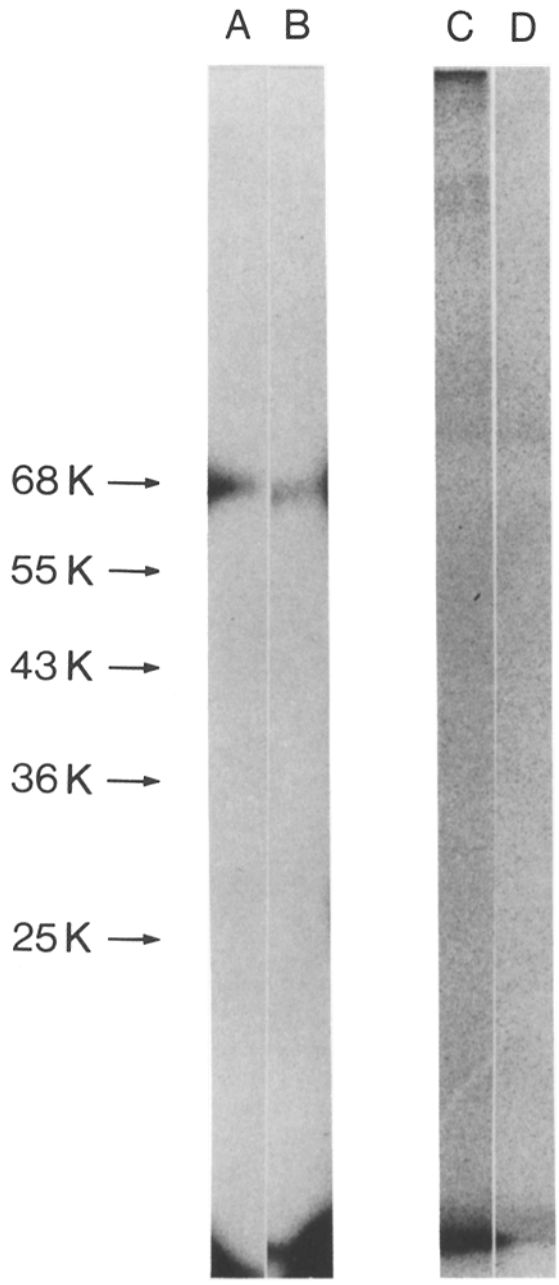


FIG. 5. 1-D gel fluorogram of $^{125}$I-labeled AEF component I after IEF and lentil lectin affinity chromatography. Flat-bed IEF fractions 17 (tracks A and C) and 19 (tracks B and D) of AEF component I (Fig. 4 A) were radioiodinated, separated on a lentil lectin column into their unbound (tracks A and B) and bound (tracks C and D) components, respectively, and then analyzed under reducing conditions by 1-D 10% SDS-PAGE. The position of migration of the molecular weight markers, bovine serum albumin (68,000), Ig heavy chain (55,000), ovalbumin (43,000), porcine lactate dehydrogenase (36,000), and Ig L-chain (25,000) are indicated by arrows.

mol wt component remains uncertain, as it appeared in both of the lectin-bound and -unbound fractions. It is possible, however, that these lectin fractions contain proteins of similar molecular size but that are either glycoprotein or nonglycoprotein in nature, respectively.

To determine whether the 68,000- and 10,000- to 15,000-mol wt components can be found in associated form in a larger molecular weight complex, $^{125}$I-labeled fractions 17 and 19 of Fig. 4 A and pooled fractions 17–19 of Fig. 4 B were subjected to electrophoresis under nonreducing conditions. For comparison, the samples were also run under reducing conditions on the same 1-D slab gel. Samples were not subjected to lentil lectin chromatography before electrophoresis. The results shown in Fig. 6 reveal that identical gel patterns were obtained when fractions 17, 19, and 17–19 were analyzed under reducing (tracks A, C, and E, respectively) and nonreducing (tracks B, D, and F, respectively) conditions. Two major bands were observed in the 68,000- and 10,000- to 15,000-mol wt regions. The bands were quite broad because the gel was overloaded with the samples to identify any possible contaminating minor components. Because no proteins >68,000 mol wt were seen, it is unlikely that the 68,000- and 10,000- to 15,000-mol wt components are covalently associated. A noncovalent association between these components may not be ruled out, however, as they were run in the presence of SDS.

To examine the possible subunit structure of the two AEF components, $^{125}$I-labeled fractions 17, 19, and 17–19 were subjected to electrophoresis under dissociating conditions on a 1-D acid-urea 10% polyacrylamide slab gel in the absence of SDS (20). Only the 68,000- and 10,000- to 15,000-mol wt components appeared on this gel (T. Delovitch. Unpublished observations.). Thus, these AEF components do not seem to be composed of noncovalently associated subunits.

The molecular heterogeneity of these components was further assessed by 2-D gel electrophoresis. The fluorogram of $^{125}$I-labeled fraction 17–19 (Fig. 4 B) shown in Fig. 7 reveals a major spot (enclosed area) having a mol wt of ~68,000 and a pI of ~5.8. This pI value is in close agreement with that previously estimated for this component by preparative IEF (Fig. 4). Moreover, the gel pattern indicates that this component is presumably homogeneous because one, and only one, spot was evident in this region of the gel. In contrast, the radioactivity that appeared in the 10,000- to 15,000-mol wt region at the dye front of the gel was not confined to a single discrete spot but was spread across the entire pH gradient. It is therefore possible that the 10,000- to 15,000-mol wt component identified above is not a single protein with biological activity, but rather that this mol wt region contains a series of products of similar size that result from the degradation of either the 68,000-mol wt component or of a protein somewhat larger in mol wt than 68,000. Alternatively, the radioactivity in the 10,000- to 15,000-mol wt regions of the gels shown in Figs. 5–7 may derive in part from residual non-protein-bound, free $^{125}$I in the samples.

Taken together, the gel analyses presented above suggest that the *H-2*-restricted helper activity of AEF is a property of a single, non-glycoprotein having a mol wt of ~68,000 and a pI of ~5.8.

*Serological Characterization.* Because the 68,000-mol wt component of AEF appears to be a single, responder T cell-derived product that recognizes Ia antigens determined by the *I-A* subregion of the stimulator haplotype, it was considered important to serologically identify the antigenic determinants on this molecule.

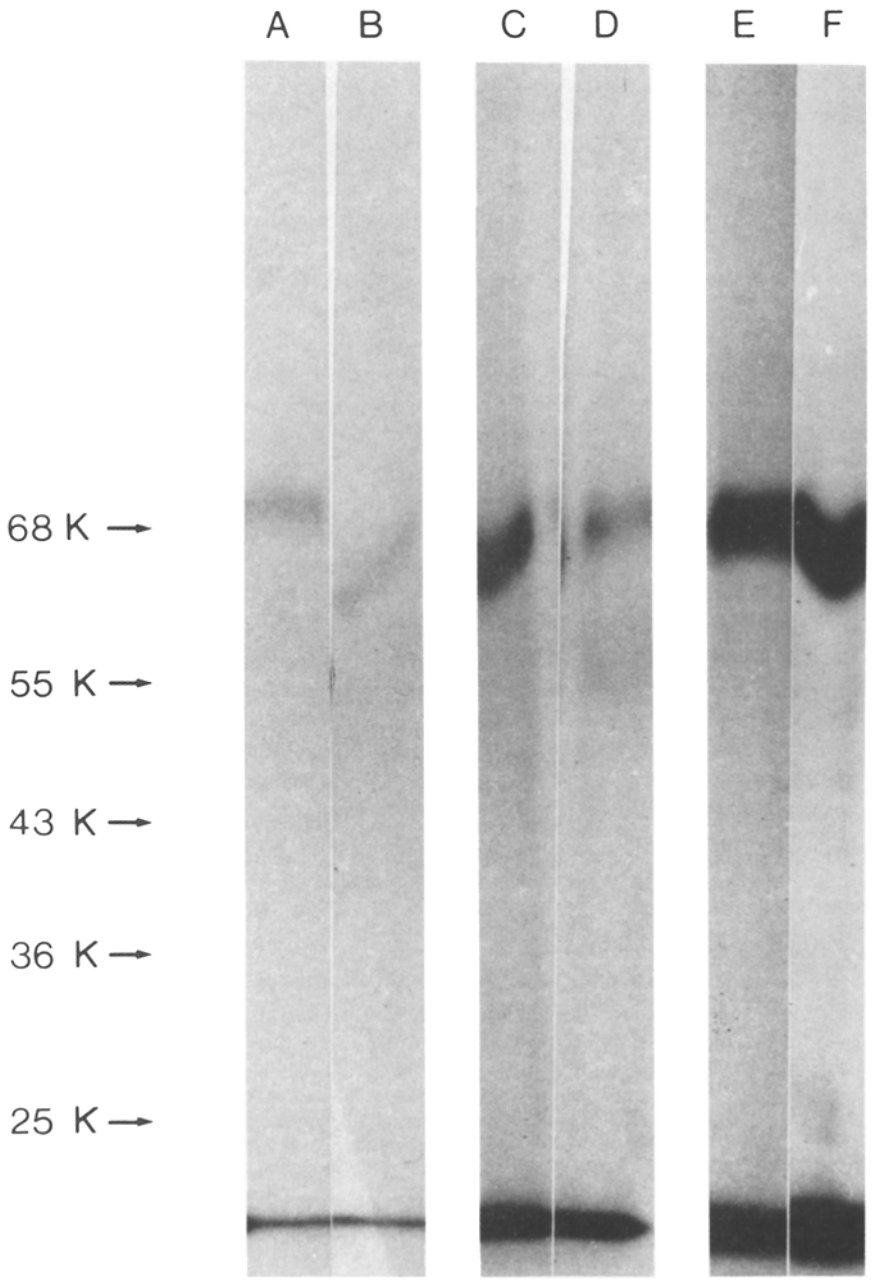


FIG. 6. 1-D gel fluorogram of $^{125}$I-labeled AEF component I after IEF. Flat-bed IEF fractions 17 (tracks A and B) and 19 (tracks C and D) of Fig. 4A and vertical-column IEF fractions 17–19 (tracks E and F) of Fig. 4B were radioiodinated and analyzed by 1-D 10% SDS-PAGE under either reducing (tracks A, C, and E) or nonreducing (tracks B, D, and F) conditions. Molecular weight markers are as in Fig. 5.

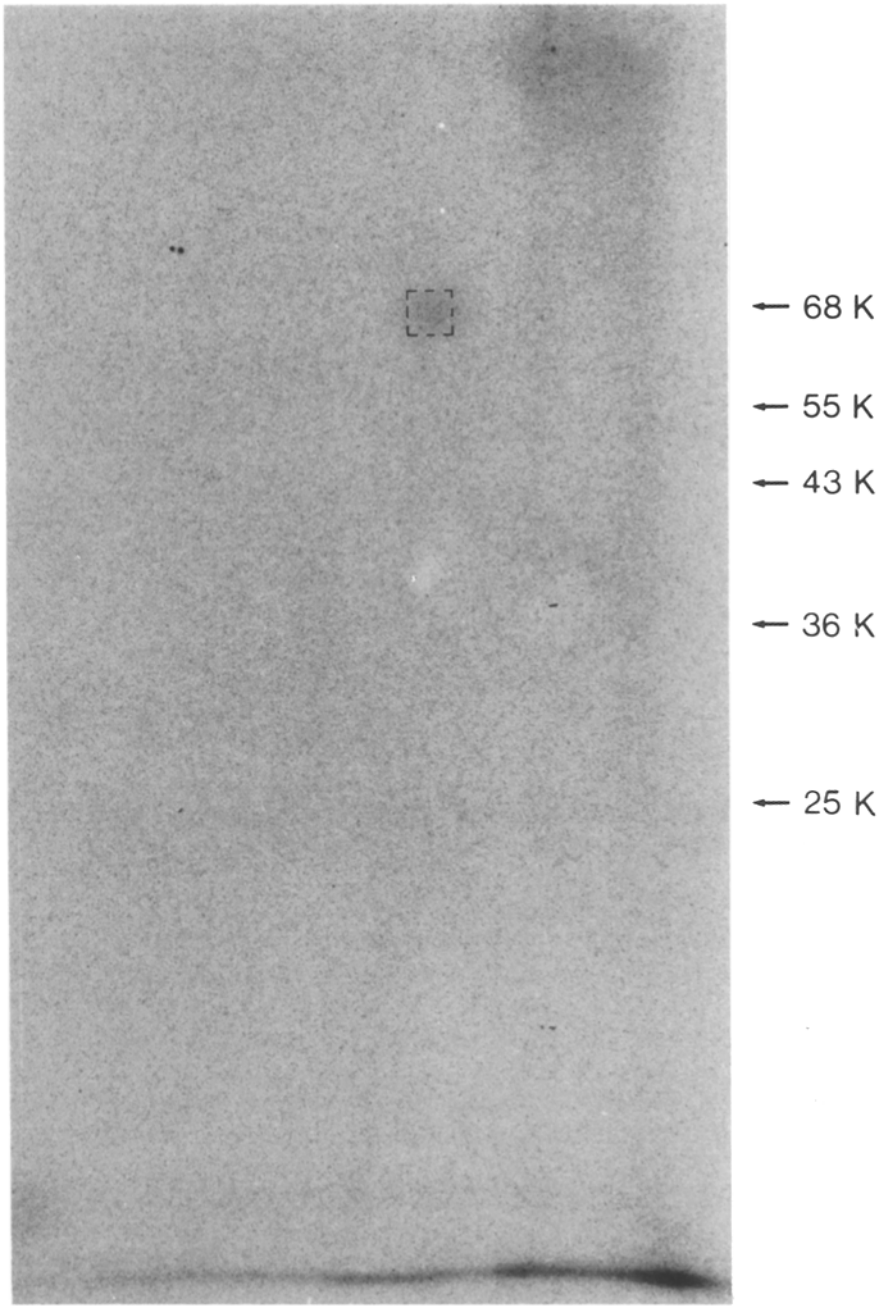


FIG. 7. 2-D gel fluorogram of $^{125}$I-labeled AEF component I after IEF. $^{125}$I-labeled samples of vertical-column IEF fractions 17–19 (Fig. 4 B) were separated by IEF in the first dimension (left to right) and by 10% SDS-PAGE in the second dimension (top to bottom). The basic end (pH 7.5) is at the left and the acidic end pH (4.5) at the right. Molecular weight markers are as in Fig. 5. The radioactive spot in the 68,000-mol wt region at pI 5.8 is enclosed.

IEF fractions 17 and 19 (see Fig. 4A) were pooled, radioiodinated, and aliquots containing ~5,000-6,000 cpm were immunoprecipitated with various antisera overnight. They were then treated for 30 min with SaCI and the pellets were counted. A similar method of serologic analysis was recently used to characterize hapten-specific T cell hybrid products (14). The results presented in Table II indicate that each of the antisera used yielded a negative reaction with the purified, 68,000-mol wt *H-2*-restricted component of AEF. Thus, within the limits of sensitivity of this radioimmunoassay, which is considered to be more direct and perhaps more sensitive than testing for biological activity of factors after immunoadsorption (see Fig. 1B), it can be concluded that the 68,000-mol wt AEF component does not bear any Ia determinants of either the stimulator or responder haplotype, does not carry Ig isotypic H-chain and L-chain determinants or Ig idiotypic determinants, and is unrelated to BSA and gp70.

## Discussion

To further probe the molecular basis for the *H-2* restriction of allogeneic lymphocyte interaction, a biological and biochemical analysis of an AEF produced across an *H-2* difference was carried out. The results presented demonstrate that AEF consists of two distinct components that possess different biochemical and biological properties (see Table III).

One component, component I, chromatographs in the 50,000- to 70,000-mol wt range by gel filtration on ACA 54 under nondissociating conditions, elutes from DEAE-Sephacel between 0.05 and 0.1 M NaCl, has a pI of ~5.8, and is not a glycoprotein as based on its inability to bind to lentil lectin. Gel electrophoretic analyses indicated that component I is comprised of a single, homogeneous protein of ~68,000 mol wt. It is rather unlikely that this protein is either mouse serum albumin

TABLE II

*Serological Characterization of AEF Component I*

| Antiserum | cpm precipitated* |
|---|---|
| A.TH anti-A.TL (anti-$I^k$) | 85 |
| (A.TH × B10.HTT)$F_1$ anti-A.TL (anti-$I\text{-}A^k$) | 46 |
| (B10 × HTI)$F_1$ anti-B10.A(5R) (anti-$I\text{-}E^k$) | 56 |
| A303 (anti-$A_e^k$:$E_\alpha^k$)‡ | 42 |
| A.TL anti-A.TH (anti-$I^s$) | 61 |
| Normal mouse serum | 52 |
| Rabbit anti-mouse $V_H$ | 79 |
| Rabbit anti-mouse $V_L$ | 85 |
| Rabbit anti-mouse $F_v$ | 65 |
| Rabbit anti-CRI | 90 |
| Rabbit anti-Thy-1 | 59 |
| Rabbit anti-BSA | 95 |
| Rabbit anti-mouse Ig | 83 |
| Normal rabbit Ig | 75 |
| Goat anti-gp 70 | 70 |
| None | 20 |

* Each reaction included 5,000-6,000 cpm of $^{125}$I-labeled AEF component I, 25 μl of antiserum, and 100 μl of SaCI. The arithmetic mean of cpm precipitated in duplicate samples is shown.

‡ Approximately 30 μg of purified A303 $IgG_{2a}$ monoclonal anti-$A_e^k$:$E_\alpha^k$ (12) was used.

TABLE III

*Biochemical, Serological, and Biological Properties of AEF*

| Property | Component I | Component II |
|---|---|---|
| Biochemical | | |
| Molecular weight (ACA 54) | 50,000–70,000 | 30,000–35,000 |
| Molecular weight (SDS-PAGE) | 68,000 | ND* |
| Salt elution from DEAE-Sephacel | 0.05–0.1 M | 0.15–0.2 M |
| pI | 5.8 | 4.3, 4.9 |
| Lentil lectin affinity | – | – |
| Serological | | |
| Ia | – | – |
| Ig (isotypic and idiotypic) | – | – |
| Biological | | |
| Helper activity | | |
| *H-2* restricted | + | – |
| *H-2* nonrestricted | – | + |
| Stimulation of CTLL growth | – | + |
| Stimulation of thymocyte mitogenesis | – | + |
| Generation of cytotoxic T cells | – | + |

* ND, not done.

(mol wt 68,000) or gp 70, the major envelope glycoprotein (mol wt ≃70,000) of murine leukemia RNA viruses. Serum albumins from a variety of species have a pI of 4.7 (21), and gp 70 is a glycoprotein that has extensive pI microheterogeneity in the pH range of 4–6 (22). The latter two proteins, therefore, differ from the AEF 68,000-mol wt component in their net charge. In addition, the 68,000-mol wt component of AEF is not immunoprecipitable by either rabbit anti-BSA or goat anti-Scripps virus gp 70 antisera (Table II). A band of 10,000–15,000 mol wt was also evident in the gel electrophoretograms of component I; however, as a result of its considerable charge heterogeneity on a 2-D gel, we tentatively concluded that this region of the gel consists primarily of either degradation products or free $^{125}I$ and not a discrete product(s) with biological activity. The latter conclusion is compatible with the observation that no AEF helper activity was found in this molecular size region upon fractionation on ACA 54. Component I possesses helper activity for $H\text{-}2^d$ nude spleen cells, for T cell-depleted spleen cells of the stimulator ($H\text{-}2^a$), but not the responder ($H\text{-}2^s$), haplotype, and for T cell-depleted spleen cells of haplotypes that share only an *I-A*-subregion identity with the stimulator haplotype. Thus, the helper activity of component I seems to be *H-2* restricted.

Component II has a mol wt of ~30,000–35,000, elutes from DEAE-Sephacel between 0.15 and 0.2 M NaCl, and does not bind to lentil lectin (data not shown). It elicits a primary anti-SRBC response of nude spleen cells and of T cell-depleted spleens of both the responder and stimulator haplotypes. Moreover, it also stimulates (*a*) the growth of a CTLL cell line in vitro, (*b*) thymocyte mitogenesis in the presence of nonmitogenic doses of Con A, and (*c*) the generation of cytotoxic T cells in either thymocyte or nude spleen cell cultures. The latter three biological activities are not manifested by component I. Furthermore, whereas the helper activity of component I is *H-2* restricted, the helper activity of component II is not *H-2* restricted. It is clear,

therefore, that AEF components I and II differ markedly in their biochemical and biological properties.

The properties described here for component II are identical to these previously reported for a class of lymphokines derived from culture supernates of Con A-activated mouse spleen cells and known as TCGF (11), or otherwise designated as either costimulator or interleukin-2 (23, 24). TCGF has pI of 4.3 and 4.9 (11) and does not bear Ia antigens (23). These findings indicate that TCGF can also be found in MLR culture supernates and appears to be a major active component of AEF. It is likely that this component is a T cell product because a factor with similar activity has recently been detected in the supernatant fluids of a Con A-stimulated T cell hybridoma (25) and TCGF was recently shown to be produced by *I-A*-negative T lymphocytes (26).

Con A-activated spleen cell supernates also contain another lymphokine of 30,000 mol wt that has a pI of 3–4, activates a nongenetically restricted anti-SRBC response of T cell-depleted spleen cells, and does not possess any of the T cell stimulatory activities common to TCGF and AEF component II (11). This lymphokine is commonly termed T cell-replacing factor (TRF) (27). It is not yet known whether AEF contains TRF-like activity. If this is the case, TRF would be a constituent of AEF component II and could be separated from the TCGF-like activity in AEF by isoelectric focusing.

A comparison may be made of the biochemical and biological properties of the AEF described above with those of another AEF, which for clarity and simplicity will be termed AEF′. Whereas AEF′ was shown by Armerding et al. (28) to consist of a 40,000- to 65,000-mol wt component when fractionated under nondissociating conditions, the AEF characterized in this study yielded 50,000- to 70,000- and 30,000- to 35,000-mol wt peaks of activity when separated under similar conditions. These AEF components did not bind to lentin lectin, whereas AEF′ did bind to lentil lectin. The DEAE-Sephacel elution profiles of both AEF′ and AEF displayed peaks of helper activity at 0.07 M NaCl and 0.14–0.2 M NaCl. Chromatography of AEF′ under dissociating conditions demonstrated that it consists of 40,000-mol wt and 12,000-mol wt components, respectively. Electrophoresis patterns of the AEF 50,000- to 70,000-mol wt peak under either nonreducing, reducing, or dissociating conditions each revealed a major band at 68,000 mol wt and a minor band at 10,000–15,000 mol wt. The relationship of the latter low molecular weight AEF component to the 12,000 dalton AEF′ component is unknown, because no biological activity was ever found to be associated with a 10,000- to 15,000-mol wt component of AEF. Similarly, no functional relationship is apparent between the 40,000- to 65,000-mol wt nondissociated form of AEF′ and the 68,000-mol wt component I of AEF. Both the nondissociated form of AEF′ and its two subunits, which act synergistically, provide helper activity for T cell-depleted spleen cells of the responder haplotype (activity for stimulator haplotype was not tested). The helper activity of AEF component I was *H-2* restricted for cells of the stimulator haplotype. Because the 30,000- to 35,000-mol wt component II of AEF is not genetically restricted, it may be functionally related to the 40,000-mol wt subunit of AEF′. Compatible with this suggestion is the recent observation that AEF′ can autonomously induce a primary CTL response in vitro in the absence of stimulating target cells during the sensitization phase (29). This AEF′ activity presumably is mediated by its TCGF-like activity which probably resides in

its 40,000-mol wt component. As shown above, the 30,000- to 35,000-mol wt component II of AEF displays TCGF-like activity. Hence, although AEF′ and AEF have some biochemical and biological properties in common, they also differ in certain other properties.

As (*a*) helper T cells interact in an *H-2*-restricted manner with both antigen and self Ia determinants on macrophages and B cells (reviewed in 1, 2, and 9) and (*b*) AEF component I is *H-2* restricted in its helper activity, the immunological significance of this 68,000-mol wt protein warrants further discussion. The biological importance of the TCGF-like AEF component II has already been discussed elsewhere (11, 30).

AEF component I is most likely a product of an alloactivated MLR responder helper T cell. Support for this cellular origin of component I derives from two main findings. First, ~90–95% of the alloactivated MLR responder cell population used to prepare AEF was serologically detected to be GVHR donor T cells. It is known that GVHR donor T cell blasts passively acquire host cell-derived *H-2* and Ia antigens onto their surface (10), a similar intercellular exchange of Ia antigens occurs from stimulator cells to responder T cells during an MLR (31). In addition, the Ly antigen phenotype of these GVHR- and MLR-activated T blasts is Lyt-$1^+$,$2^-$ (10, 31), which is identical to the Ly antigen phenotype of antigen-specific helper T cells (32). These data previously prompted us to propose that this cellular transfer of Ia antigens is responsible in part for the *H-2* restricted interactions of antigen- and alloantigen-specific helper T cells (10). Note that TCGF also requires the presence of Lyt-$1^+$,$2^-$ T cells for its production (26), and hence AEF components I and II may be products of the same T cell subpopulation. Second, component I possesses helper activity and seems to recognize Ia antigens of the GVHR host and MLR stimulator haplotype. It is, therefore, conceivable that component I is a GVHR donor and MLR responder T cell surface membrane protein that binds to host and stimulator Ia antigens, and is present in AEF because it is either shed or secreted by the activated MLR responder T cells. If this is indeed the case, then this 68,000-mol wt component of AEF might be classified as a T cell alloantigen receptor.

AEF is commonly regarded as an antigen-nonspecific helper factor. However, because AEF component I exhibits alloantigen specificity, AEF should now be considered to be an alloantigen-specific helper factor. 9 out of 10 AEF examined thus far elicit *H-2*-restricted helper activity (Table I). It is probable that these AEF each consist of a structurally distinct 68,000-mol wt component that has binding specificity for different Ia determinants. As a corollary, this might infer the existence of a large repertoire of polymorphic T cell receptors for *H-2*-linked alloantigens. The observation that the helper activity of AEF component I is about twofold greater than that of component II (Fig. 2) might explain why most AEF preparations when tested at an appropriate dilution manifest *H-2*-restricted help. We suggest that the *H-2*-restricted or non-*H-2*-restricted activity of a particular AEF is dependent upon its relative amounts of component I and II identified herein.

Based on immunoadsorption studies, we have previously reported that various AEF are Ia-positive helper factors (4–7, 9). These Ia antigens were shown to be stimulator B cell and/or macrophage derived. In the present study, it is important to note that although a similar immunoadsorption experiment indicates that AEF bears stimulator haplotype derived Ia antigens (Fig. 1), a more direct and sensitive serological assay of AEF component I suggests that this *H-2*-restricted component is Ia-negative (Table

II). Furthermore, spots corresponding to Ia antigens are absent from the 2-D gel fluorogram of component I (Fig. 7). It is possible that stimulator cell-derived Ia antigens are active components of AEF and can perhaps bind noncovalently to component I of AEF when AEF is in an unfractionated form. A biochemical resolution of AEF into its various components may, as a result of extensive manipulation, cause the dissociation of Ia antigens from the 68,000-mol wt component of AEF. Nevertheless, because various anti-Ia sera do not bind to this 68,000 component, this result would tend to negate the possibility that such antisera contain anti-idiotype antibodies that bind to the anti-self Ia idiotype of T cell-derived factors (33).

Although we have proposed that AEF component I recognizes Ia antigens determined by the $I\text{-}A^k$ subregion, the reason for its helper activity for BALB/c ($H\text{-}2^d$) nude spleen cells was unclear. The model of two-gene complementation for Ia antigens (34) might explain this result. The $H\text{-}2^k$ and $H\text{-}2^d$ haplotypes express Ia antigens composed of an $\alpha$-chain subunit ($E_\alpha$) controlled by a gene in *I-E* that is noncovalently associated with a $\beta$-chain subunit ($A_e$) controlled by a gene in *I-A*. $A_e^k$ and $A_e^d$ possess ~40–50% structural homology, whereas $E_\alpha^k$ and $E_\alpha^d$ share ~90% structural homology (35). In certain cases, helper T cells recognize an antigen in association with an $A_e$:$E_\alpha$ complex on the surface of an antigen-presenting macrophage (36). In addition, some clones of alloreactive T cells appear to recognize, and are stimulated by, the surface form of the $A_e$ chain (37). Thus, the 68,000-mol wt component of AEF may have a binding capacity for membrane-bound $A_e^k$ and $A_e^d$ chains of macrophages and/or B cells in nude spleen and T cell-depleted spleen cell populations. More significantly, if this type of interaction can be shown to occur preferentially for macrophage-derived Ia antigens, this would provide strong support for the claim that *H-2* restriction of lymphocyte interaction occurs primarily between T cells and accessory cells (macrophages) and not between T cells and B cells (38).

Our previous observations that Ia antigens determined by only the stimulator haplotype and not the responder haplotype (5–8) led us to postulate that AEF is a B cell and/or macrophage product. The data presented herein, however, suggest that the H-2-restricted activity of AEF is conferred not by Ia antigens themselves, but rather by an Ia-negative, and possibly $V_H$- and idiotype-negative, alloactivated T cell-derived, 68,000-mol wt protein that can recognize and bind to Ia antigens on B cells and/or macrophages. This AEF component may in fact bear $V_H$ and idiotypic determinants that differ from those detected by the sera used. It seems that a ligand: receptor interaction, which is Ia:anti-Ia in nature, is mediated by AEF component I. We therefore now reinterpret our original observations to suggest that whereas AEF Ia antigens may be products of B cells and macrophages, the Ia-negative, *I-A*-restricted component of the AEF analyzed here is an alloactivated T cell product.

During the last few years, a number of soluble T cell-derived helper and suppressor factors have been described. Although their active components were not purified to apparent homogeneity, their biological activity was ascribed to molecules in the 40,000- to 70,000-mol wt range. More recently, with the availability of the hybridoma and T cell cloning technologies, products of both T cell hybrids formed by the fusion of a thymoma cell line with antigen-specific suppressor T cells (14) and of nontransformed, antigen-specific suppressor T cell clones (39) have been characterized. Some of these molecules have been shown to have a mol wt of either 62,000 (14) or 68,000 (39) and to express (14, 40) or not express (39) Ia determinants. Another antigen-

specific suppressor factor derived from uncloned T cells, when purified, was shown to have a mol wt of 68,000 and to be Ia negative (41). Although none of these T cell products bear any known Ig isotypic H or L chain determinants, one of them seems to carry cross-reactive idiotypic determinants (14). The AEF component I purified and characterized here is similar in size (68,000 mol wt) to the above-mentioned T cell products, does not possess Ia determinants, and bears neither $V_H$, $C_H$, L-chain, nor idiotypic determinants. This AEF component bears some resemblance to a previously identified 70,000-mol wt, Ia-negative, idiotype-positive constituent of alloactivated T cells (42). More extensive biochemical and biological analyses of the above-mentioned, as well as other, T cell-derived products should ultimately resolve the molecular nature of antigen-specific and alloantigen-specific T cell receptors. They may even provide an answer the problem of whether dual recognition by T cells is mediated by either two receptors—one receptor for foreign antigens and a second receptor for *H-2* antigens—or a single receptor for these two groups of antigens.

## Summary

An allogeneic effect factor (AEF) derived from mixed lymphocyte reaction (MLR) cultures of alloactivated A.SW (*H-2*$^s$) responder T cells and irradiated A/WySn (*H-2*$^a$) stimulator spleen cells helps an in vitro primary anti-erythrocyte plaque-forming cell PFC response of BALB/c nude spleen cells and also A/WySn but not A.SW T cell-depleted spleen cells. AEF activity is adsorbed by anti-*I*$^k$ and anti-*I-A*$^k$ but not by anti-*I-J*$^k$, anti-*I-EC*$^k$, and anti-*I*$^s$. Gel filtration of ACA 54 resolves AEF into two main components that which appear in the 50,000- to 70,000-mol wt (component I) and 30,000- to 35,000-mol wt (component II) regions, respectively. Component I has a mol wt of 68,000, elutes from DEAE-Sephacel at 0.05–0.1 M NaCl, and has an isoelectric point (pI) of 5.8. It helps A/WySn but not A.SW B cells and, therefore, is *H-2* restricted. Component II is not *H-2* restricted, because it helps both A.SW and A/WySn B cells. It also stimulates (*a*) the growth of a long-term cytotoxic cell line in vitro, (*b*) Con A-induced thymocyte mitogenesis, and (*c*) the generation of cytotoxic T cells. The latter three properties of component II are not shared by component I. In addition, component II elutes from DEAE-Sephacel at 0.15–0.2 M NaCl and has a pI of 4.3 and 4.9. Ia determinants and Ig $V_H$, $C_H$, L-chain, and idiotypic determinants are not present on either component I or component II. The properties of component II are identical to that of a T cell growth factor produced by Con A-stimulated spleen cells. It is suggested that the *H-2*-restricted component I of AEF might be an MLR-activated responder T cell-derived Ia alloantigen receptor.

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