ROLE OF Ia ANTIGENS IN GRAFT VS. HOST REACTIONS

II. Molecular and Functional Analysis of T Cell Alloreactivity by the Characterization of Host Ia Antigens on Alloactivated Donor T Cells*

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The genetic and biochemical basis for the inclusion of T cell alloreactivity within the immunological repertoire of T helper (T_H)[1] lymphocytes remains a perplexing problem (1, 2). Recent evidence (3–9) suggests that the ability of T_H cells to bind to molecular complexes formed on antigen-presenting cells between a nominal antigen and either self or nonself (allo) Ia molecules regulates their different phenotypic patterns of immune responsiveness. This type of regulation implies that antigen presentation markedly influences the T_H cell repertoire (10). It is likely that this repertoire is governed by the diversity and specificity of T_H cell membrane receptors for epitopes of the nominal antigen and either self- or allo-Ia molecules. Because self- and allo-Ia molecules express not only private epitopes but also public epitopes (11–13), it is conceivable that their T_H cell-derived self-Ia and allo-Ia complementary receptors possess some structural similarities (reviewed in 1, 14). This proposed structural homology of different Ia receptors might enable a self-Ia receptor to bind with high affinity to a self-Ia molecule and with lower affinity to an allo-Ia molecule; the reverse is expected for an allo-Ia receptor. It is apparent, therefore, that a better understanding of the biological relationship between T cell self-reactivity and allo-reactivity should emerge from a study of the binding capacity of alloactivated T cells for self-Ia and allo-Ia molecules.

Using immunofluorescence, we have previously shown (15, 16) that during a graft vs. host reaction (GVHR), Lyt-1^+2^- donor T cell blasts bind detectable amounts of allo-Ia molecules of host origin but not self-Ia molecules of donor origin. Host-derived

* Supported by grant MT-5729 from the Medical Research Council of Canada and a grant from the National Cancer Institute of Canada.

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[1] *Abbreviations used in this paper:* AEF, allogeneic effect factor; C′, rabbit complement; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; GVHR, graft-vs.-host response; LPS, *Escherichia coli* bacterial lipopolysaccharide; MLR, mixed lymphocyte culture response; NMS, normal mouse serum; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; PFC, plaque-forming cell; PVC, polyvinylchloride; RAMFab, rabbit anti-mouse Fab(IgG); SaCI, *Staphylococcus aureus* Cowan I strain; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SRBC, sheep erythrocyte; T_H, T helper cell.

I-A alloantigens were more readily transferred to donor T cells than were host *I-E* alloantigens. These data suggested that in vivo alloactivated T cells do not synthesize Ia antigens but rather acquire onto their surface Ia antigens from allogeneic antigen-presenting cells, perhaps via an interaction with their allo-Ia receptors. To explore these possibilities, it was of interest to extend and quantitate these observations using anti-*I-A* and anti-*I-E* antibodies in radioimmune cell binding assays and immunoprecipitation assays of unlabeled and radiolabeled activated donor T cell Ia antigens, respectively. The functional significance of an intercellular transfer of Ia antigens during a GVHR posed another intriguing problem. In particular, we wished to determine whether different subpopulations of alloactivated donor T_H cells that were separated by immunofluorescence according to their capacity to bind host-derived *I-A*^{k} alloantigens were *I-A*^{k} restricted in their ability to elicit antibody production by either donor haplotype B cells or host haplotype B cells. Such analyses could conceivably bear directly on the functional relationship of self-reactive and alloreactive T_H cells.

In this report, we demonstrate that GVHR-activated donor T cells do not synthesize Ia antigens of the donor *H-2*^{s} haplotype but do acquire, in vivo, A_α^k, A_β^k, A_e^k, and E_α^k Ia polypeptide chains from *H-2*^{a} host cells. We also show that the *I-A*-restricted helper activity of sorted subpopulations of activated donor T_H cells assayed in an in vitro primary antibody response correlates closely with their in vivo acquired binding capacity for either donor (self)- or host (allo)-*I-A* products. This genetic restriction occurs upon interaction with only nonimmune B cells and not with immune B cells. The requirements for T_H cell self-Ia and allo-Ia antigen receptor occupancy are discussed in terms of their role both in T_H cell repertoire development and B cell activation. Some of the data reported in this manuscript have been presented in preliminary form elsewhere (17, 18).

Materials and Methods

Mice. Strains A.SW/SgSn (*H-2*^{s}) and A/WySn (*H-2*^{a}) were purchased from The Jackson Laboratory, Bar Harbor, Maine. All other strains were inbred and maintained in our mouse colony at the University of Toronto.

Antisera. The hyperimmune alloantisera (A.TH × C3H.OL)F_1 anti-C3H/DiSn (anti-*K*^{k}), (A.TH × B10.HTT)F_1 anti-A.TL (anti-*I-A*^{k}, *I-J*^{k}), B10.A(3R) anti-B10.A(5R) (anti-*I-J*^{k}), (B10 × HTI)F_1 anti-B10.A(5R) (anti-*I-E*^{k}), A.TH anti-A.TL (anti-*I*^{k}), (BALB.B × A.AL)F_1 anti-A.TL (anti-*K*^{s}), and A.TL anti-A.TH (anti-*I-A*^{s}) were produced as previously described (19). $F(ab')_2$ of rabbit anti-mouse Fab(IgG) was kindly supplied by Dr. M. Letarte (Department of Medical Biophysics, University of Toronto, Canada). Fluorescein-conjugated $F(ab')_2$ of goat anti-mouse IgG was purchased from N. L. Cappel Laboratories, Cochranville, Pa.

Hybridomas. A303 is a hybridoma cell line that secretes a monoclonal antibody that detects an Ia molecule whose α-chain (E_α) is encoded by either *I-E*^{k} or *I-E*^{d} and whose β chain (A_e) is encoded by *I-A*^{k} (20). The Y17 monoclonal antibody used detects an A_e^k:E_α^k Ia molecule (21) and was kindly provided by Dr. E. A. Lerner (Department of Pathology, Yale University, New Haven, Conn.). The anti-Ia.7 monoclonal antibody used detects an *I-E*^{k} molecule (22) and was kindly provided by Dr. U. Hammerling (Sloan Kettering Institute for Cancer Research, New York). The 10-2.16, 10-3.6, and 11-5.2 hybridomas, each of which secrete a monoclonal anti-*I-A*^{k} antibody (23) and the H0.13-4 cell line, which secretes a monoclonal anti-Thy-1.2 antibody (24), were generously provided by Dr. L. A. Herzenberg (Department of Genetics, Stanford University, Stanford, Calif.) and Dr. M. Gefter (Department of Biology, Massachusetts Institute of Technology, Boston, Mass.) to the Salk Institute Cell Distribution Center, La Jolla, Calif., which was our source for these reagents. These antibodies were purified from ascites fluids using protein A-Sepharose CL 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.,

Piscataway, N. J.) affinity chromatography (25). Biotinylated A303 was prepared by Dr. M. L. Phillips (Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada) according to Lerner et al. (21), and biotinylated-11-5.2 was purchased from Becton-Dickinson Monoclonal Antibody Center, Mountain View, Calif.

Preparation of GVHR-alloactivated T Cells. Alloactivated donor T cells were obtained as described (15). Briefly, 10^8 donor thymocytes from mice (4–8 wk old) were injected i.v. into irradiated (800 rad), *H-2* congenic, and incompatible recipients (8–15 wk old) of matched sex. Recipient spleen cells were recovered 5 d layer. Splenic T cell blasts were selected by Ficoll-Hypaque gradient centrifugation and then panned (26) for Ig^- cells using the IgG fraction of a polyvalent goat anti-mouse Ig serum (27) at 2.5 μg/ml to coat the panning dishes during 90 min at 23°C. Spleen cells were panned for 70 min at 4°C. The surface-antigen phenotype of the nonadherent Ig^- cell fraction was determined by microcytotoxicity with either H0.13-4 monoclonal anti-Thy-1.2 antibody or anti-K^s and anti-K^k alloantisera and EDTA agarose-adsorbed rabbit complement (C′); the percent of Ig-bearing cells in this cell fraction was determined by immunofluorescence using fluorescein-conjugated $F(ab')_2$ of goat anti-mouse IgG and a Leitz Orthoplan fluorescent microscope (E. Leitz, Inc., Rockleigh, N. J.) (15).

Radioimmune Cell-binding Assay. The direct binding of mouse Ig to formalin-fixed lymphocytes was evaluated by an indirect cell binding assay as described (20). Formalin-fixed target cells (3×10^5 to 5×10^5) were incubated with an appropriate concentration range (10^{-2} to 10^{-5} vol/vol dilution of ascites) of monoclonal anti-$I\text{-}A^k$ antibody for 15 h at 4°C in polyvinyl chloride (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, Va.) U-bottomed 96-well microtiter plates to determine the antibody concentration required to achieve saturation binding. The binding buffer used was phosphate-buffered saline (PBS) containing 10% fetal calf serum (FCS), 0.1% gelatin, 10 mM Tris (pH 7.5), and 0.02% Na azide. Plates were washed three times and ^{125}I-protein A was added for 1 h at 4°C. After a further three washes and drying of the plates, ^{125}I cpm bound per well was determined in a Beckman 300 gamma counter (Beckman Instruments Inc., Fullerton, Calif.) at 70–80% efficiency. Background binding was defined by the binding of ^{125}I-protein A at each concentration of monoclonal antibody used to appropriate normal spleen cells and normal thymocytes. ^{125}I-protein A was diluted before use in 0.25% gelatin, 10 mM Tris (pH 7.5), and 0.02% Na azide.

To relate the observed ^{125}I-protein A specific binding to a relative Ig concentration, a standard titration curve was generated for each monoclonal antibody used. Serial dilutions of ascites of each of the monoclonal antibodies were incubated for 16 h at 4°C with acrylic plastic microtiter plate wells previously coated with affinity purified $F(ab')_2$ of RAM Fab (20). After three washes of the plate, ^{125}I-protein A was added for 1 h at 4°C under the same conditions as for the cell binding assays. The results of this solid-phase binding established an empirical relationship of the form $y = x^s$, where y is the ^{125}I-protein A specifically bound cpm, x is the relative antibody concentration, and s is the limiting slope of the binding curve. The validity of this method of interpreting the ^{125}I-protein A cpm bound in cell binding assays was explored by evaluating the binding to suspensions containing varying amounts of expected positive and negative prototype splenic lymphocytes. In this manner, the ^{125}I-protein cpm specifically bound was found to vary linearly with the ratio of positive:negative spleen cells used (J. F. Harris and T. L. Delovitch, manuscript in preparation).

Radiolabeling, Immunoprecipitation, and Gel Electrophoresis. The donor A.SW (*H-2*^{s}) and host A/WySn (*H-2*^{a}) strains were used in these GVHR analyses. [^{35}S]Methionine-labeled, Nonidet-P40 (NP-40) solubilized, lentil-lectin column-bound and eluted glycoprotein-containing lysates of either normal spleen cells or GVHR-activated donor T cells were prepared as previously described (28). Aliquots of unlabeled, NP-40 solubilized, glycoprotein-containing lysates obtained from GVHR-activated donor T cells were adjusted to pH 8.5 using 1 M phosphate buffer, pH 9, and then labeled with ^{125}I using the Bolton-Hunter reagent (1375 Ci/mmole specific activity; Amersham Corp., Oakville, Ontario). This was performed according to the method of Langone et al. (29), with the exception that a PBS buffer containing 0.1% gelatin and 10 mM Tris-HCl, pH 7.4, was used, as reported earlier (20, 30).

Samples (100 μl) containing ~1×10^6 to 5×10^6 cpm were reacted with 25–50 μl of various antisera for 18 h at 4°C and were further treated for 30 min at 4°C with 250 μl of a 10% (vol/vol) heat-killed, formalin-fixed suspension of *Staphylococcus aureus*, Cowan I strain (SaCI). The

pelleted SaCI were washed four times with 1 ml of PBS that contained 0.5% NP-40, 2 mM methionine, and 5 mM KI. SaCI-bound proteins were eluted and electrophoresed either under reducing conditions for 16 h at 10 mA on a one-dimensional sodium dodecyl sulfate (SDS)-polyacrylamide (10% wt/vol) slab gel or on a two-dimensional gel according to O'Farrell (31), as described (30). Gels were impregnated with EN^{3}HANCE (New England Nuclear, Boston, Mass.), dried, and then exposed to preflashed (32) Kodak XR-1 film (Eastman Kodak Co., Rochester, N. Y.) using a Cronex Lightning Plus image-intensifying screen (Du Pont Canada Inc., Markham, Ontario). The ^{125}I-radioactivity in one-dimensional gel protein bands was quantitated by scanning the autoradiograms at 520 nm with a Joyce Loebl microdensitometer (Joyce, Loebl & Co., Gateshead, England). Alternatively, 2-mm gel slices were cut from a one dimensional gel, their [^{35}S]methionine radioactivity eluted during 16 h at 37°C into 0.5 ml of 0.1% SDS, and then quantitated in the presence of 5 ml Aquasol (New England Nuclear) using a Searle Mark III liquid scintillation counter (Searle Radiographics Inc., Des Plaines, Ill.).

Cell Sorting. The donor A.SW ($H\text{-}2^s$) and host A/WySn ($H\text{-}2^a$) strains were used in these GVHR analyses. GVHR-activated donor ($H\text{-}2^s$) T cell blasts were obtained by Ficoll-Hypaque centrifugation and panning for Ig$^-$ cells as indicated above. After washing in sterile PBS containing 5% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 40 μg/ml gentamycin, and 0.02% Na azide (PBS and 5% FCS), ~30×10^6 donor T cells were pelleted and then resuspended in 30 μl of biotinylated-anti-$I\text{-}A^k$ (11-5.2, 1 mg IgG/ml) adjusted to about 450 μl with PBS and 5% FCS and incubated for 30 min at 4°C. Cells were then centrifuged through 100% sterile, heat-inactivated FCS and washed once with PBS and 5% FCS (15), treated with 60 μl of fluoresceinated-avidin (2.5 mg/ml, Becton-Dickinson & Co., Rutherford, N. J.) for a further 30 min at 4°C, and washed as above. Alternatively, in control samples, 1×10^6 donor T cells were either left unstained, stained with only 2 μl of fluoresceinated-avidin, stained with 4 μl of biotinylated-anti-A_e^k:E_α^k (A303, 0.6 mg IgG/ml) and 2 μl of fluoresceinated-avidin, or stained with 10 μl of the IgG fraction (10 mg IgG/ml) of anti-$I\text{-}A^s$ and 20 μl of fluoresceinated-F(ab')$_2$ of goat anti-mouse F(ab) (0.1 mg F(ab')$_2$/ml). The fluorescence and sorting of scatter-gated viable cells was determined with Dr. R. O. Miller's OCI (Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, Toronto, Canada) fluorescence-activated cell sorter (FACS) (33). Cell suspensions to be used subsequently for tissue culture were maintained aseptically both before and during FACS sorting.

Functional Assay of FACS-sorted T Cells. GVHR-activated donor ($H\text{-}2^s$) T cells were sorted with the FACS into subpopulations that had either acquired ($I\text{-}A^{k\oplus}$) or had not acquired ($I\text{-}A^{k\ominus}$) host Ia antigens. Approximately 10^5 cells of each sorted T cell subpopulation were cocultured with ~4×10^5 HO-13-4 plus C'-treated spleen cells of either donor or host origin in the absence or presence of 10^6 sheep erythrocytes (SRBC) (batch 5 of SRBC, obtained from Woodlyn Laboratories, Guelph, Ontario, was used in all of these experiments). T-depleted spleen cells were obtained from either nonimmune mice or mice that were immunized 5 d previously intraperitoneally with 0.1 ml of a 20% (vol/vol) suspension of SRBC. Control cultures contained either no T cells, unsorted activated donor T cells, or a mixture of equal numbers (10^5) of $I\text{-}A^{k\oplus}$ and $I\text{-}A^{k\ominus}$ sorted donor T cells to examine any mutually suppressive effects of these subpopulations. Triplicate cultures in 0.21 ml of RPMI 1640 medium (Grand Island Biological Co., Burlington, Ontario) containing 5% FCS, 4 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin, and 40 μg/ml gentamycin were set up in flat bottomed Falcon 3040 Micro Test II 96-well microtiter plates (Fisher Scientific, Don Mills, Ontario) that were maintained in a stationary form for 5 d at 37°C in a 5% CO_2 humidified incubator. Direct (IgM) and indirect (IgG) plaque-forming cells (PFC) were enumerated as before (34) using the Cunningham and Szenberg slide method (35).

Results

Donor T Cell Origin of GVHR-activated Blasts. The surface antigen phenotype of the panned Ig$^-$ fraction of GVHR-activated, radioresistant, blasts was analyzed in part by dye exclusion microcytotoxicity and immunofluorescence (Table I). Most of the Ig$^-$ blasts were shown to be T cells because ~95% of these blasts were lysed by the HO.13-4 monoclonal anti-Thy-1.2 antibody plus C'. Only 4% of the selected Ig$^-$

TABLE I

*Surface Antigen Phenotype of GVHR-activated Blasts**

Group	Serum treatment	Antibody specificity	Percent lysis‡
1	HO.13-4	Anti-Thy-1.2	95 ± 3
2	Fluoresceinated $F(ab')_2$ of goat anti-mouse IgG	Anti-IgG	4 ± 1§
3	(BALB.B × A.AL)F_1 anti-A.TL	Anti-K^s	93 ± 2
4	(A.TH × C3H.OL)F_1 anti-C3H/DiSn	Anti-K^k	4 ± 2

* In this GVHR response, the donor A.SW (*H-2*^{s}) and host A/WySn (*H-2*^{a}) strains were used.

‡ The values shown in groups 1, 3, and 4 are cytotoxicity indices that are normalized for variations in background control lysis (5–12%). They were obtained in three different experiments by a dye exclusion microcytotoxicity assay and are presented as the arithmetic SEM. A cytotoxic index is calculated as

$$\frac{\text{percent specific lysis} - \text{percent control lysis}}{100\% - \text{percent control lysis}}.$$

§ The value in group 2 represents the percentage of T cell blasts that were stained with fluoresceinated-anti IgG. The arithmetic SEM obtained in two immunofluorescence experiments is shown.

blasts were found by immunofluorescence to be residual Ig-bearing cells. The T blasts were further shown to have a predominant donor origin because ~93% and 4% of the cells were lysed by treatment with C′ and anti-K^s (anti-donor) and anti-K^k (anti-host), respectively. An approximate donor:host distribution ratio of these T blasts is therefore 93:4 = 23:1. Such a ratio is within expectation for GVHR-activated T blasts obtained from an irradiated host environment because most of the cells that home to and repopulate the spleen during 5 d postirradiation (800 rad) of the host would be expected to be of donor origin. Furthermore, we have not detected any back-stimulation in an in vitro MLR response of radioresistant, host-derived, responder T cells by donor-derived, irradiated (3,000 rad) stimulator spleen cells (19, 34, unpublished observations). The latter result tends to minimize any potential contribution of a reverse in vivo allogeneic effect to the generation of a significant number of GVHR-activated host-derived T cell blasts. Thus, the data presented in Table I suggest that the major proportion of the GVHR-activated blasts characterized in this study are donor T cells.

Quantitation of Host Ia Molecules on Donor T Cells. Our immunofluorescence studies previously demonstrated that host Ia antigens encoded by the *I-A* subregion but neither the *I-J* nor *I-E* subregion are transferred from the surface of irradiated host cells to the surface of GVHR-activated donor T cells. However, they did not indicate (*a*) the number of host *I-A* molecules acquired by donor T cells and (*b*) whether the transfer of *I-J* and *I-E* products, and *I-E* products in particular, were undetectable for quantitative rather than qualitative reasons. Radioimmune cell-binding assays, which are much more sensitive and quantitative than immunofluorescence, were carried out, therefore, to estimate the relative number of host-derived *I-A* and *I-E* alloantigens that bind to donor T cells.

Activated donor T cells were generated in GVHR responses of either BALB.B ($H\text{-}2^b$) → BALB.K ($H\text{-}2^k$), BALB.K → BALB.B, B10 ($H\text{-}2^b$) → B10.BR ($H\text{-}2^k$), or B10.BR → B10 donor → host combinations. They were reacted with either the 10-2.16 or the 10-3.6 monoclonal anti-$I\text{-}A^k$ antibody and ^{125}I-protein A in an indirect cell-binding assay. These antibodies bind specifically to splenic lymphocytes of the $H\text{-}2^k$ haplotype but not the $H\text{-}2^b$ haplotype (Table II, group 3) and thus were considered appropriate to study the intercellular exchange of $I\text{-}A^k$ alloantigens during an $H\text{-}2^b$ → $H\text{-}2^k$ GVHR response.

Table II shows that both 10-2.16 and 10-3.6 bound specifically (0.5–1.5% binding) to BALB.B → BALB.K- and B10 → B10.BR-activated donor T cells but not to BALB.K → BALB.B- and B10.BR → B10-activated donor T cells (groups 1 and 2). No significant binding above control values (~0.1% binding) was detected in the latter two combinations; this suggests that the Ig^+ cells that comprised 3–5% of these donor T cell populations did not give rise to any specific binding. Neither did any significant binding occur to A.TL (I^k) normal resting thymocytes (group 3). Because the amount of radioactivity bound to unseparated activated spleen cells was very similar to that of Ig^- panned activated spleen cells (groups 1 and 2), it is likely that most if not all of the observed binding was contributed by the Ig^- T cell blasts in

TABLE II

*Estimation of Relative Number of Host I-A-coded Ia Antigens on Activated Donor T Cells**

Group	Target cell Donor → Host	^{125}I-Protein A bound‡	
		10-2.16§ (anti-$I\text{-}A^k$)	10-3.6 (anti-$I\text{-}A^k$)
		cpm	
1	BALB.B ($H\text{-}2^b$) → BALB.K ($H\text{-}2^k$)		
	Unseparated activated spleen cells	116 ± 28	50 ± 18
	Ig^- panned activated spleen cells	160 ± 15	83 ± 8
	BALB.K → BALB.B		
	Unseparated activated spleen cells	33 ± 7	29 ± 13
	Ig^- panned activated spleen cells	35 ± 9	20 ± 7
2	B10 ($H\text{-}2^b$) → B10.BR ($H\text{-}2^k$)		
	Unseparated activated spleen cells	358 ± 136	350 ± 21
	Ig^- panned activated spleen cells	394 ± 36	335 ± 64
	B10.BR → B10		
	Unseparated activated spleen cells	54 ± 7	38 ± 13
	Ig^- panned activated spleen cells	58 ± 13	36 ± 10
3	BALB.K Normal spleen cells	872 ± 130	600 ± 58
	BALB.B Normal spleen cells	39 ± 5	28 ± 9
	A.TL Normal thymocytes	44 ± 11	29 ± 6

* The binding of mouse Ig to formalin-fixed lymphocytes (5×10^5 cells/sample) was evaluated as described in Materials and Methods. Mean background binding (at saturating levels of binding to BALB.K spleen target cells) was 41 ± 22 cpm for 10-2.16 and 32 ± 17 cpm for 10-3.6. Binding to target cells with one SD of this basal binding was defined as background.

‡ Mean binding of ^{125}I-protein A (2.3×10^4 cpm = 5 ng added per sample) in cpm ± SE. The ^{125}I-protein A used was labeled with the Bolton-Hunter reagent (29). Five observations were made for each value.

§ The specificity of the monoclonal 10-2.16 and 10-3.6 antibodies used is indicated in parentheses. See Materials and Methods for more details.

TABLE III

*Estimation of Relative Number of Host I-A- and I-E-coded Ia Antigens on Activated Donor T Cells**

Group	Target cell Donor → Host	^{125}I-Protein A bound‡		
		Y17§ (anti-*I-E*^{k})	anti-Ia.7 (anti-*I-E*^{k})	11-5.2 (anti-*I-A*^{k})
		cpm		
1	A/SW → A/Wy	160 ± 36 (0.04)	265 ± 13 (0.16)	300 ± 40 (0.03)
2	B10 → B10.BR	236 ± 7 (0.09)	252 ± 11 (0.16)	707 ± 58 (0.12)
3	B10.BR → B10.S	102 ± 10 (0.02)	106 ± 10 (0.04)	169 ± 13 (0.01)
4	B10 Thymus	38 ± 8 (0)	37 ± 7 (0)	52 ± 8 (0)
5	B10.S Spleen	123 ± 36 (0.02)	157 ± 4 (0.08)	151 ± 5 (0.01)
6	B10.BR Spleen	1270 ± 34 (1.0)	910 ± 30 (1.0)	3450 ± 65 (1.0)

* The conditions of this experiment are the same as those in Table II except that ^{125}I-protein A (3×10^4 cpm = 0.45 ng) was added to each sample for 1 h at 20°C. All cell preparations used (3×10^5 cells/sample) were unseparated activated spleen cells.

‡ Mean binding of ^{125}I-protein A in cpm ± SE. Four observations were made for each value. The figures in parentheses represent the relative number of cell surface bound Ig molecules. These numbers were obtained by reference to a standard titration curve of monoclonal antibody bound to F(ab')$_2$ of RAM Fab as described in Materials and Methods. The relationship between ^{125}I-protein A cpm bound (y) and relative antibody concentration (x) determined from the slope of this titration curve is $y = x^{0.7}$. For each monoclonal antibody used, the value obtained for the binding to B10.BR spleen cells was normalized to 1.0, and the binding to B10 thymocytes was normalized to 0.

§ The specificity of the monoclonal Y17, anti-Ia.7, and 11-5.2 antibodies used is indicated in parentheses. See Materials and Methods for more details.

these activated cell populations. It is apparent, therefore, that T cells must be alloactivated to acquire the capacity to bind detectable amounts of nonself Ia antigens. These data confirm our immunofluorescence studies (15) and provide stronger evidence that alloactivated donor T cells do not express Ia antigens of the donor haplotype but do bind *I-A* alloantigens of the host haplotype.

A comparison of the binding data presented in groups 1 and 2 of Table II vs. that of group 3 also shows that activated donor T cells bind approximately one-fifth to one-half as many ^{125}I-protein A cpm when compared to a normal host spleen cell (2.6–3.8% binding). Our previous studies suggest that a resting B cell expresses ~10^4 to 10^5 Ia molecules on its surface (J. Harris, unpublished observations). Thus, if it is assumed that (*a*) the number of surface Ia molecules expressed is linearly related to the ^{125}I-protein A cpm bound at the saturating concentration of monoclonal anti-*I-A*^{k} used and (*b*) the kinetics used result in the binding of one protein A molecule to one

molecule of mouse IgG_{2a} antibody, we estimate that between 2×10^3 and 5×10^4 host *I-A*-encoded Ia molecules may be bound by a GVHR-activated donor T cell. Due to uncertainties associated with the estimation of both the number of antibody molecules bound at saturation and the protein A:antibody stoichiometric ratio at any given point in a binding curve (Table III), the absolute number of host Ia molecules found on donor T cells might be overestimated by as much as twofold to threefold. Thus, although the estimated absolute number of transferred host Ia molecules might be somewhat inaccurate, the observed relative proportion of host Ia molecules on host spleen cells (presumably B cells) and activated donor T cells is quite significant.

To determine whether the intercellular exchange of *I-E* alloantigens occurs during a GVHR response, Y17 (anti-A_e^k:E^k) and anti-Ia.7 (anti-$I\text{-}E^k$) hybridoma antibodies were reacted in an indirect cell-binding assay to A.SW($H\text{-}2^s$) and B10($H\text{-}2^b$) donor T cells that were activated in response to A/WySn($H\text{-}2^a$) and B10.BR($H\text{-}2^k$) host alloantigens, respectively. These reagents bind to splenic lymphocytes of the $H\text{-}2^a$ and $H\text{-}2^k$ haplotypes but not to the $H\text{-}2^s$ and $H\text{-}2^b$ haplotypes (21, 22). For comparison, 11-5.2, a monoclonal anti-$I\text{-}A^k$ antibody that binds to $H\text{-}2^k$ lymphocytes and not to $H\text{-}2^s$ and $H\text{-}2^b$ lymphocytes (23) was also used. It is evident from Table III that host *I-E* products are indeed transferred to donor T cells during a GVHR. To relate the observed ^{125}I-protein A cpm bound to a relative Ig concentration, it was necessary to derive an empirical relationship between these parameters. Using this relationship, the relative proportion of *I-E* molecules transferred (4–16%) is about the same as the number of *I-A* molecules transferred (3–12%). It is also apparent, however, that the relative number of $I\text{-}A^k$ determinants detected on a B10.BR spleen lymphocyte is about threefold to fivefold greater than the number of $I\text{-}E^k$ determinants. Thus, both *I-A*- and quantitatively fewer *I-E*-encoded host Ia molecules are transferred from host cells to GVHR-activated donor T cells. This quantitative difference is presumably the reason why the transfer of host *I-E* molecules was previously undetected by the less sensitive immunofluorescence assay (15).

Immunochemical Analysis of Donor T Cell Ia Antigens. To extend the cell-binding data presented above, it was of interest to analyze immunochemically whether both or perhaps only one of the α-chain and β-chain subunits of host-derived *I-A* and *I-E* alloantigens are transferred to donor T cells. It was also of interest to ascertain whether any structural alterations of *I-A* and *I-E* Ia polypeptides occur during intercellular transfer. If such a modification(s) in structure was observed, it might provide a better understanding of the role of Ia antigens in the genetic control of lymphocyte interaction.

Immunoprecipitates of ^{125}I-labeled glycoprotein-enriched fractions of panned Ig^- donor T cell blast NP-40 lysates were analyzed under reducing conditions by one-dimensional and two-dimensional gel electrophoresis. Anti-Ia alloantisera were used for these experiments because they were more plentiful than the relevant monoclonal antibodies at the time; these two sources of antibodies yield identical gel profiles (36). Fig. 1 illustrates that A/WySn ($H\text{-}2^a$) host I^k-derived (Fig. 1 A) but not A.SW ($H\text{-}2^s$) donor I^s-derived (Fig. 1 D) Ia molecules were precipitated from the donor T cell lysates. Both Ia α-chain (31,000–34,000 mol wt) and β-chain (28,000–31,000 mol wt) polypeptides of host-derived $I\text{-}A^k$ (Fig. 1 B) and $I\text{-}E^k$ (Fig. 1 C) Ia alloantigens were transferred from host cells to donor T cells. Because the basic I_i invariant chain (31,000 mol wt) usually found in association with Ia molecules is not well-labeled

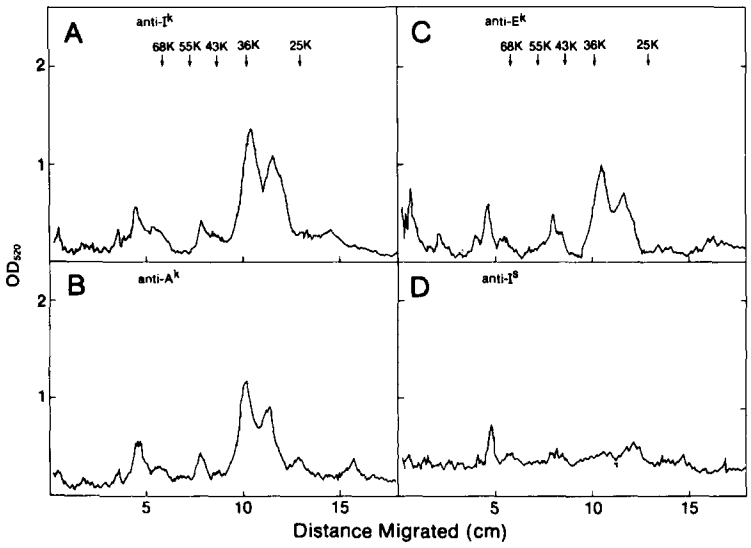

FIG. 1. Host (I^k) Ia antigens on donor (I^s)-activated T cells. One-dimensional gel fluorograms of immunoprecipitated ^{125}I-labeled host A/WySn Ia antigens from activated donor A.SW T cells. ^{125}I-labeled, glycoprotein-enriched, NP-40 lysates of A.SW-activated T cells were precleared with NMS and SaCI and then immunoprecipitated with either (A) A.TH anti-A.TL (anti-I^k), (B) (A.TH × B10.HTT)F_1 anti-A.TL (anti-A^k), (C) (BIO × HTI)F_1 anti-B10.A (5R) (anti-E^k), or (D) A.TL anti-A.TH (anti-I^s), using SaCI. Samples were analyzed by 10% SDS-PAGE under reducing conditions for 16 h at 10 mA. Microdensitometer tracings of autoradiograms of different tracks of a slab gel exposed by fluorography are shown. Migration positions of mol wt markers bovine serum albumin (68,000), IgG H-chain (55,000), ovalbumin (43,000), porcine lactate dehydrogenase (36,000) and IgG L-chain (25,000) are indicated.

with ^{125}I (37, 38), we did not detect this polypeptide in any of the gel profiles shown in Fig. 1, despite the fact that we used a gel of sufficient length (18 cm) to permit its resolution (38). It is therefore not yet known whether I_i can also undergo intercellular exchange or whether it is an integral membrane protein that cannot be shed or secreted.

A comparison was made of the two-dimensional gel spot patterns obtained for anti-I^k immunoprecipitates of ^{125}I-labeled glycoprotein-containing fractions of lysates of normal host spleen cells (Fig. 2A) and activated donor T cells (Fig. 2B). Identical patterns, which revealed the presence of intact A_α, A_β, A_e (E_β), and E_α polypeptides (36) were obtained for these samples. This confirmed the transfer of host I-A^k and I-E^k molecules to donor T cells, as shown in Fig. 1 above. More interestingly, the data demonstrated that no detectable structural alteration of host Ia molecules occurred during their transfer and binding to donor T cells, i.e., they retained their same apparent size and net charge. No Ia polypeptides were immunoprecipitated from the control samples treated with either anti-I^s (Fig. 2C) or normal mouse serum (NMS) (Fig. 2D).

The question of whether murine T cells that have either not been activated or have been stimulated to proliferate by either an antigen, alloantigen, or mitogen stimulus synthesize Ia antigens has elicited a controversial and still inconclusive answer (reviewed in 18, 39). We addressed this question further by examining the synthesis of Ia antigens in [^{35}S]methionine labeled GVHR-activated donor T cells. The one-

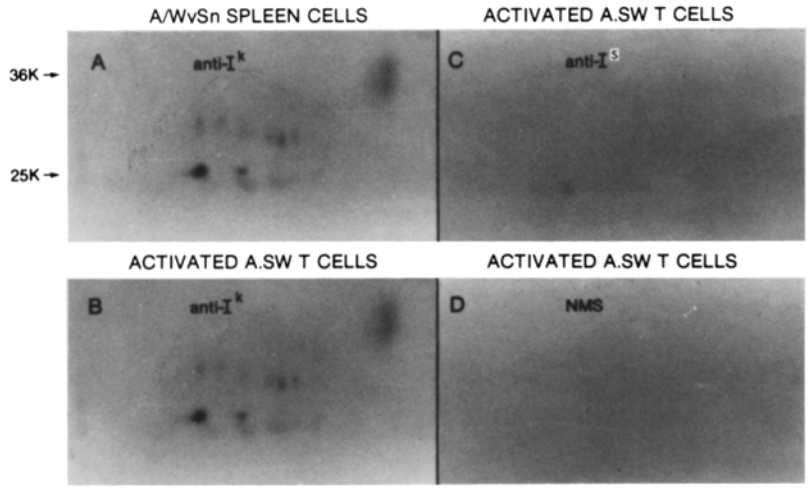

FIG. 2. Two-dimensional gel fluorograms of immunoprecipitated ^{125}I-labeled host A/WySn Ia antigens. ^{125}I-labeled, glycoprotein-enriched, NP-40 lysates were prepared from either normal A/WySn (*H-2*^{a}; *I-A*^{k}, *I-E*^{k}) spleen cells (A) or donor A.SW (*H-2*^{a}; *I-A*^{s}, *I-E*^{s}) GVHR-activated T cells (B–D). Precleared samples were reacted with either (A, B) A.TH anti-A.TL (anti-*I*^{k}), (C) A.TL anti-A.TH (anti-*I*^{s}), or (D) NMS. Samples were separated by isoelectric focusing 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) is at the right. Mol wt markers are as in Fig. 1. Only the 20,000–40,000 mol wt portions of the fluorograms are shown.

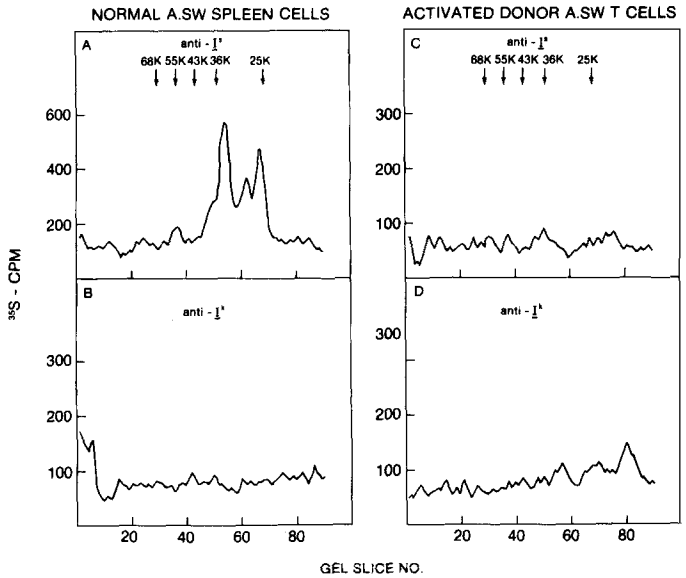

FIG. 3. One dimensional gel electrophoresis of immunoprecipitated [^{35}S]methionine-labeled donor A.SW Ia antigens. NP-40 lysates enriched in glycoproteins were prepared from [^{35}S]methionine-labeled normal A.SW spleen cells (A, B) or GVHR-activated donor A.SW T cells (C, D). Precleared samples were treated with either (A, C) A.TL anti-A.TH (anti-*I*^{s}) or (B, D) A.TH anti-A.TL (anti-*I*^{k}). The 10% SDS-slab gel was run under reducing conditions, and the radioactivity in 2-mm gel slices was determined. Mol wt markers are as in Fig. 1.

dimensional gel profiles presented in Fig. 3 clearly show that, in contrast to the synthesis of Ia^s antigens by normal A.SW spleen cells (Fig. 3A), activated donor A.SW T cells do not synthesize immunoprecipitable Ia^s antigens (Fig. 3C). In the control samples, no synthesis of Ia^k antigens was detected by these cell populations (Fig. 3B and 3D), as expected. These immunochemical data are quite compatible with the immunochemical and cell-binding data summarized above and the immunofluorescence data reported earlier (15).

Note also that Ia α- and β-chains and the I_i invariant chain were immunoprecipitated by anti-I^s from A.SW normal spleen cells (Fig. 3A). These Ia polypeptides are presumably $I\text{-}A^s$ products because no $I\text{-}E^s$ Ia antigens are detectable by immunoprecipitation (36). The reason for the detection of the synthesis of I_i in this experiment and not in Figs. 1 and 2 above is most likely due to the fact that it can be easily labeled with [^{35}S]methionine but not with ^{125}I (38; D. Charron, Department of Virology and Tumor Immunology, Hospital Cochin, Paris, France, personal communication).

Functional Analysis of Donor T Cell Subpopulations. The studies of alloantigen binding to GVHR-activated donor T cells pose several important questions with regards to the functional capacity of these cells. First, if the binding of host Ia molecules to GVHR-activated donor Lyt-1^+2^- T cells occurs via specific donor T cell allo-Ia receptors, does this postulated Ia:anti-Ia ligand:receptor type of interaction regulate the helper T cell activity of these cells in I-region restricted immune responses? Second, do subpopulations of GVHR donor T cells exist that recognize only self (donor)-Ia or only allo (host)-Ia, and if so, do they display an H-2 preference in their ability to help B cells of either the donor or host haplotype, respectively? Third, does the repertoire of a self-Ia-reactive helper T cell overlap with that of an allo-Ia-reactive helper T cell? Functional analyses of GVHR-activated donor T cells that either bind or do not bind host Ia molecules were carried out in an attempt to answer some of these questions.

Donor A.SW ($H\text{-}2^s$) → A/WySn ($H\text{-}2^a$) GVHR-activated T cell blasts were prepared by Ficoll-Hypaque gradient centrifugation and panning. Approximately 95% of these cells were lysed with either a monoclonal anti-Thy-1.2 antibody (H0.13-4) or an anti-K^s serum in a microcytotoxicity assay, suggesting that they were predominantly of donor T cell origin (Table I). In initial analytical FACS experiments, aliquots (1×10^6 cells) of this cell population were reacted first with either biotinylated-anti-$I\text{-}A^k$ (11-5.2) or biotinylated-anti-A_e^k:E_α^k (A303), or both, and second with fluoresceinated-avidin. Alternatively, in control samples, cells were stained with either the IgG fraction of anti-$I\text{-}A^s$ and fluoresceinated-F(ab') of goat anti-mouse IgG, or with only the latter reagent. Fig. 4A shows that ~20% of the viable cells were specifically stained with 11-5.2 and ~16% of the cells were stained with A303. Only 80% as many donor T cells acquire host *I-E* alloantigens when compared with the number of donor T cells that acquire host *I-A* alloantigens. Approximately 20% of the cells were stained when treated with both 11-5.2 and A303, i.e., no additive effect was observed. It seems, therefore, that during this type of GVHR response, all donor T cells that bind host *I-E* molecules also bind host *I-A* molecules, and ~20% of the host-*I-A*-binding donor T cells do not bind host *I-E* products. Only ~2–3% and 3–5% of the cells analyzed were found to express $I\text{-}A^s$ antigens and to be Ig^+, respectively (Fig. 4B). These values presumably reflect the number of residual donor B cells in the cell

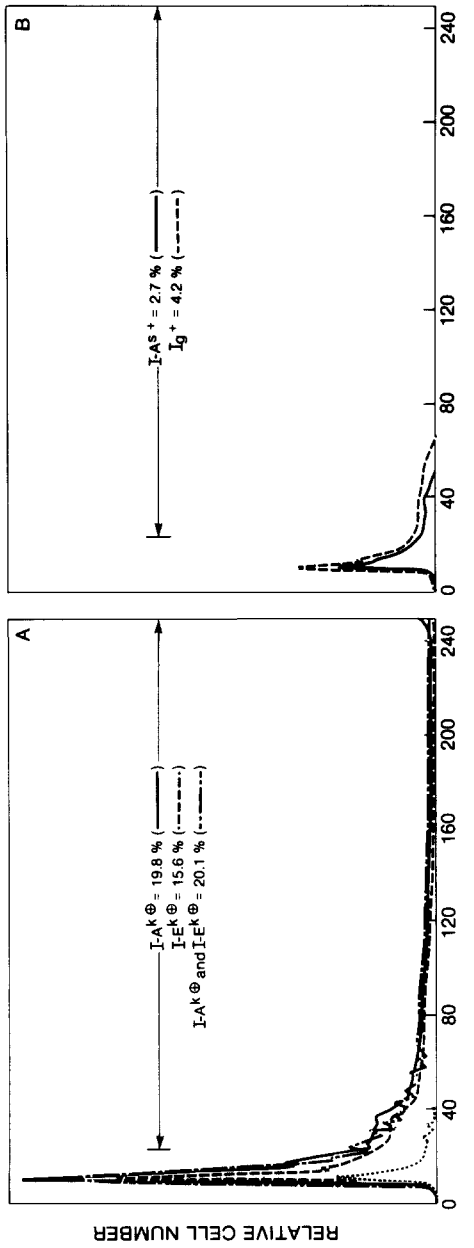

FIG. 4 A and B. See legend on facing page.

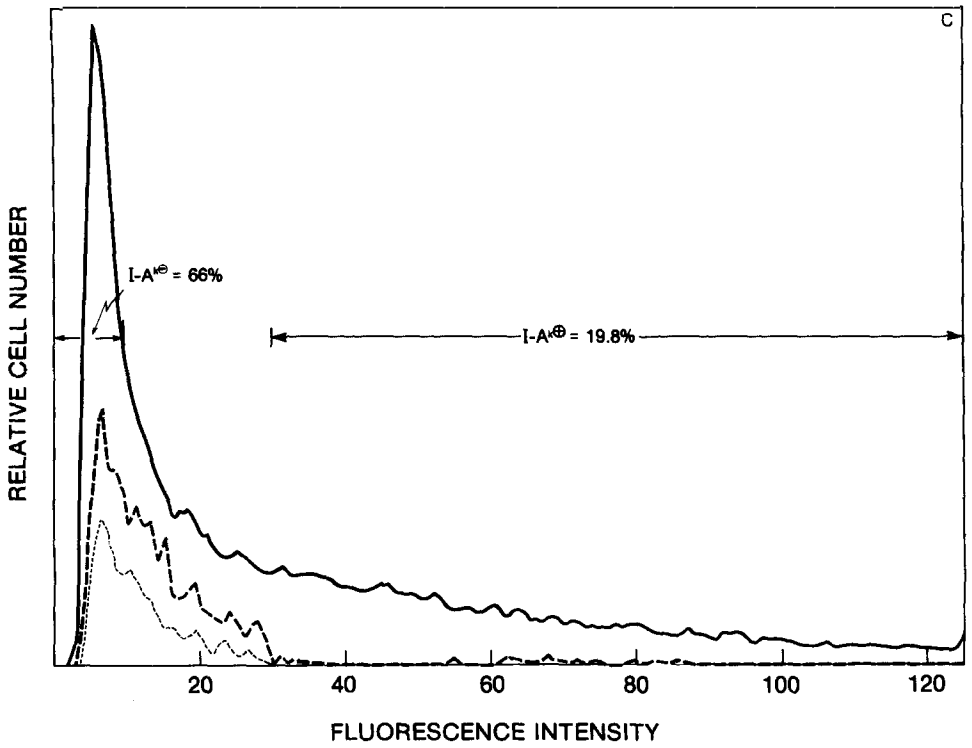

FIG. 4. FACS analysis of activated donor A.SW T cells. Donor A.SW-activated T cell blasts obtained during a GVHR from the spleens of irradiated A/WySn hosts were prepared by Ficoll-Hypaque gradient centrifugation and panning. Aliquots (1×10^6 cells) of this cell population were then stained (A), using fluoresceinated-avidin in the second step (-----) and either biotinylated-11-5.2 (——), biotinylated-A303 (- - -), or biotinylated-11-5.2 plus biotinylated-A303 (- — -) in the first step; and (B), using the IgG fraction of anti-*I-A*^{s} and fluoresceinated-F(ab')$_2$ of goat anti-mouse IgG (——) or only the latter reagent (- - -). For each aliquot, 20,000 cells were analyzed to yield the profiles shown. In (C), 30×10^6 activated A.SW donor T cell blasts were sorted after staining with biotinylated-11-5.2 and fluoresceinated-avidin (——). Approximately 20% of the brightest viable cells were taken to be *I-A*$^{k\oplus}$; the dimmest viable cells showing no fluorescence below channel 10 (~66% of cells) were taken to be *I-A*$^{k\ominus}$. Control samples (2×10^6 cells) were treated with the IgG fraction of anti-*I-A*^{s} and fluoresceinated-F(ab')$_2$ of goat anti-mouse IgG (- - -) of left unstained (autofluorescence control, ····).

population that is highly enriched for activated donor T cells.

For the functional analyses, 30×10^6 A.SW → A/WySn-activated donor T cell blasts were stained with biotinylated-11-5.2 and fluoresceinated-avidin and then sorted with the FACS (Fig. 4 C). The 20% brightest viable cells were taken to be *I-A*$^{k\oplus}$ cells, i.e., donor T cells that bound host *I-A*-encoded Ia antigens. The dimmenst 66% of viable cells showing no specific fluorescence were taken to be *I-A*$^{k\ominus}$, i.e., donor T cells that did not bind host *I-A* antigens.

The helper cell activity of *I-A*$^{k\oplus}$ and *I-A*$^{k\ominus}$ donor T cells for nonimmune T cell-depleted spleen cells of either donor or host origin was examined in an in vitro primary anti-SRBC PFC response (Fig. 5). No significant responses were observed in the control cultures in which T cells were absent (groups 1–4). Unsorted activated donor T cells helped in a PFC response of host B cells but not donor B cells (groups 5–8). *I-A*$^{k\ominus}$ donor T cells that did not bind host *I-A* antigens preferentially helped B

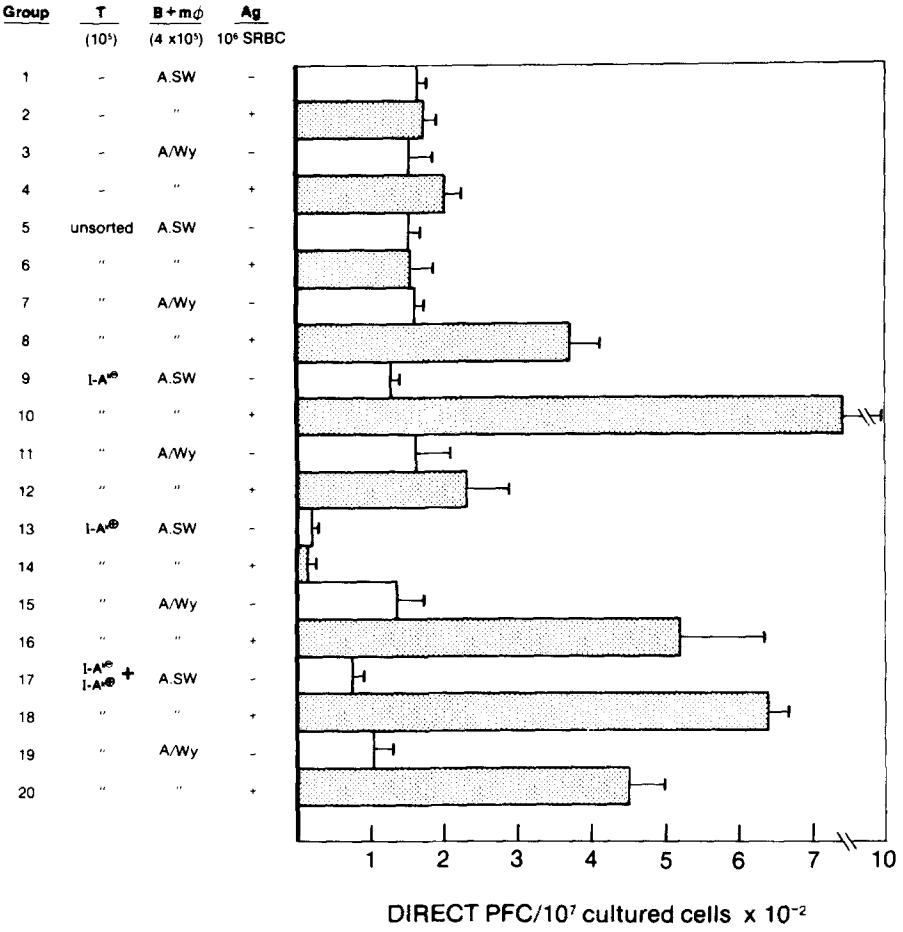

FIG. 5. H-2-restricted interaction of FACS-sorted GVHR-activated donor T cells with nonimmune B cells. GVHR-activated donor A.SW T cells were sorted (see Fig. 4C) into subpopulations that either bind ($I\text{-}A^{k\oplus}$) or do not bind ($I\text{-}A^{k\ominus}$) host Ia antigens. Their respective helper activities were assayed, with T cell-depleted spleen cells (B + Mϕ) obtained from either nonimmune A.SW or A/WySn mice in the presence or absence of SRBC used as antigen (Ag). Direct (IgM) PFC are presented as the arithmetic SEM. Data from two experiments are shown.

cells of the donor haplotype (groups 9–12). $I\text{-}A^{k\oplus}$ donor T cells that bound host *I-A* antigens helped B cells of only the host haplotype (groups 13–16). Positive responses were elicited by both donor and host haplotype-derived B cells in cultures that contained both $I\text{-}A^{k\ominus}$ and $I\text{-}A^{k\oplus}$ donor T cells (groups 17–20). No suppressive effect was evident, therefore, upon mixing these two subpopulations of donor T cells, yet donor B cells were unresponsive when interacted with unsorted donor T cells (groups 5 and 6). Although the reason for the latter result is not understood, it is possible that some donor haplotype-specific suppressor T cells were deleted from the selected sorted T cell subpopulations. In addition, it is likely that in the unsorted activated donor T cell population the precursor frequency of allo (host)-reactive T cells is much greater than that of self (donor)-reactive helper T cells. This might also account for the observed *I*-region-restricted helper activity of unsorted donor T cells for host B cells.

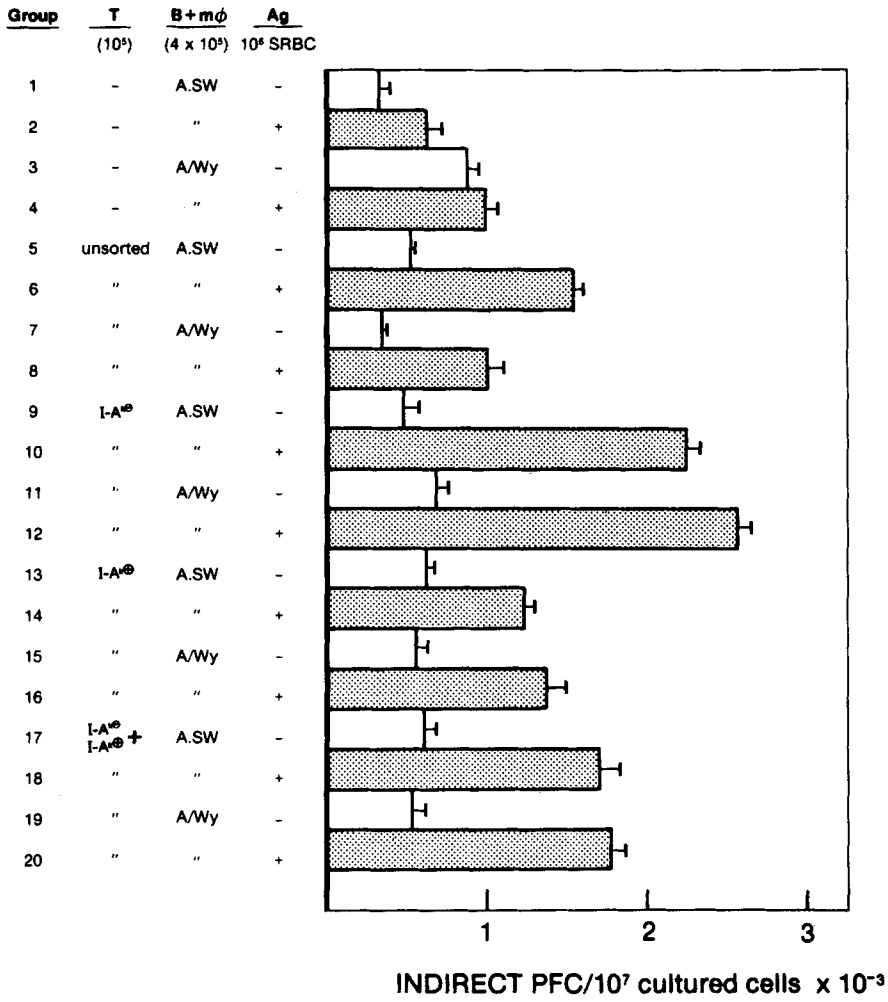

FIG. 6. H-2-nonrestricted interaction of FACS-sorted GVHR-activated donor T cells with immune B cells. The *I-A*$^{k\oplus}$ and *I-A*$^{k\ominus}$ subpopulations of donor T cells were separated as in Fig. 4C. Their helper activities for T cell-depleted spleen cells obtained from A.SW and A/WySn mice previously immunized with SRBC were assayed. Indirect (IgG) PFC are presented as the arithmetic SEM. Direct PFC (≤200) have been subtracted. Data from two experiments are shown.

When the helper activity of *I-A*$^{k\ominus}$ and *I-A*$^{k\oplus}$ donor T cells for T cell-depleted spleen cells obtained from SRBC-immunized mice was examined, each T cell subpopulation cooperated with antigen-primed B cells of the donor and host haplotypes (Fig. 6, groups 9–16). A similar finding was noted for the helper activity of unsorted donor T cells (groups 5–8). Again, no suppressor T cell activity was observed in cultures containing a mixture of the *I-A*$^{k\ominus}$ and *I-A*$^{k\oplus}$ donor T cell subpopulations (groups 17–20). Thus, whereas specific recognition of Ia antigens is required for an interaction between GVHR-activated donor T helper cells and nonimmune B cells during an IgM PFC response (Fig. 5), no I-region restricted interactions occur between alloactivated donor T helper cells and immune B cells during an IgG PFC response (Fig. 6).

Discussion

To further unravel the enigma of the genetic and molecular basis of T cell alloreactivity, a biochemical and biological analysis of GVHR-activated T lymphocytes was carried out. In particular, the Ia antigen phenotype and the helper activity of alloactivated donor T cell subpopulations in *I*-region-restricted responses were analyzed. Immature donor T lymphocytes, i.e., thymocytes, were used as the source of T cells in these studies so that their acquisition of alloreactivity could be examined in vivo during differentiation in an allogeneic host environment.

Both radioimmune cell-binding and immunoprecipitation assays demonstrated that alloactivated donor T lymphocytes do not express detectable amounts of Ia antigens of the donor haplotype but do bind readily detectable amounts of *I-A*- and *I-E*-encoded Ia antigens of the host haplotype. Interestingly, about three to five times more host *I-A* alloantigens than *I-E* alloantigens are acquired by donor T cells. As many as 2×10^3 and perhaps even as great as 5×10^4 host *I-A* molecules were estimated to be transferred to the surface of an activated donor T cell blast. This suggests that between 10^3 and 10^4 host *I-E* molecules may be bound by a donor T cell blast. Because it is likely that host Ia molecules bind to a donor T cell via an interaction with its Ia alloantigen receptors, it is intriguing to speculate whether the estimated number of host Ia molecules bound to an activated donor T cell reflects the expression of an equivalent number of Ia-receptor molecules present on such a cell. If it is assumed that one and only one Ia molecule can bind to its complementary T cell Ia receptor, then an alloreactive T cell might express between 10^3 and 5×10^4 allo-Ia receptors on its surface. It should be recognized that the estimated numbers of transferred Ia molecules and allo-Ia receptors on alloactivated T cells presented above serve only to identify the range of sensitivity of detection of our assays and might not reflect the absolute number of these molecules expressed on the surface of an activated T cell for reasons stated earlier (see Results).

The finding that fewer *I-E*- than *I-A*-encoded host Ia molecules are transferred to donor T cells is consistent with our immunofluorescent data, which indicate that there exist two subpopulations of host Ia-binding donor T cell blasts (Fig. 4A). One subpopulation binds both host *I-A* and *I-E* products; the second subpopulation binds only host *I-A* products; thus, the percentage of donor allo-*I-A*-binding T cells is greater than the percentage of donor allo-*I-E*-binding T cells. These observations might explain those reported in previous (40–43) functional analyses of GVHR responses, which showed that an *I-A* incompatibility induces a much stronger response than that obtained in an *I-E* incompatibility. They are also compatible with the results that the rate of shedding and/or secretion of *I-A* alloantigens from spleen cells is about fivefold greater than that of *I-E* alloantigens (R. Cone, Department of Pathology, Yale University, personal communication). Finally, they agree with the notion that both *I-A*- and *I-E*-encoded Ia molecules mediate antigen presentation to T cells (6, 7). Because the recognition of only syngeneic *I-E* antigens is sufficient to elicit T cell proliferative responses to certain antigens under two *Ir* gene control, by analogy it will prove interesting in the future to determine whether a subpopulation of GVHR-activated donor T cells that binds only host allo-*I-E* antigens can be identified.

It has been amply documented that different subpopulations of mouse, human, and guinea pig T cells possess either an Ia^- or Ia^+ surface antigen phenotype. The question of whether Ia^+ T cells synthesize the Ia molecules that appear on their

surface has, however, been a controversial issue. Only a limited number of direct biosynthesis studies have been carried out to address this question. Whereas some reports have suggested that nonactivated thymocytes and peripheral T cells of the mouse synthesize Ia antigens (44, 45), other reports have shown this definitely not to be the case (46, 47). Conflicting data related to the expression of Ia antigens by activated T cells has also been published. In one instance (48), the synthesis of Ia antigens by human MLR-activated responder T lymphocytes has been convincingly shown; in other instances (49) it is purported that an intercellular transfer of Ia antigens from antigen-presenting cells to responding T cells can account for a large extent of the Ia^+ phenotype of the two types of human T cells implicated in either various disease states or in mitogen, antigen, and alloantigen stimulation. In the mouse, numerous examples of this type of intercellular transfer of Ia antigens have been found. About 20% of GVHR donor T cell blasts (Fig. 4), and in other *H-2* incompatibilities as many as 30–50% of donor T cell blasts (15), acquire an Ia^+ phenotype by their ability to bind allo-Ia molecules presumably produced by host macrophages and/or B cells. Both of the latter cell types synthesize Ia antigens (46). Our biosynthetic studies in this report demonstrate that murine GVHR-activated donor T helper cells do not synthesize Ia antigens (Fig. 3). Similarly, it is known that ~30–50% of MLR-activated murine responder T cells can acquire either allogeneic or syngeneic stimulator cell-derived Ia antigens onto their surface (50, 51). Recently, it has also been shown in radiation-induced bone marrow-derived chimeric mice that ~20% of donor bone marrow-derived thymocytes bind host Ia antigens on their surface as they mature in the host thymic environment (52). T helper cells can also adsorb macrophage-derived syngeneic Ia antigens onto their surface (53–55); this adsorption is mediated by ~20% of immune T helper cells, is antigen-specific, and is dependent on the presence of $I\text{-}A^+$ antigen-presenting macrophages (54). The reason(s) for the discrepancy in the proof of synthesis, or lack thereof, of Ia antigens by different T cell subsets remains unknown. Nevertheless, in the several cases of T cell acquisition of Ia antigens cited, it is probable that an intercellular exchange of Ia molecules is mediated by the binding of Ia molecules to specific Ia antigen T cell receptors, as discussed above. It is interesting to note that both Ia α- and β-polypeptides can be transferred to T cells in an apparently intact, nonprocessed and structurally unaltered form (Figs. 1 and 2). This might result from the fact that Ia molecules are constitutively synthesized under physiological conditions, and, therefore, they need not be processed, as does a nominal antigen, before binding to T cells.

The notion of complementarity between cell surface antigens and cell surface receptors of interacting networks of lymphocytes is currently of prime importance (56). It follows that the phenomenon of Ia intercellular exchange described in this report might reflect the binding specificity of certain T cell subsets for syngeneic and/or allogeneic Ia antigens and control their respective repertoires in *I*-region-restricted responses via complementary anti-Ia:Ia interactions. That this is indeed the case is suggested by our functional analyses, which showed that the Ia antigen-binding specificity of the $I\text{-}A^{k\oplus}$ and $I\text{-}A^{k\ominus}$ subpopulations of GVHR-activated donor T cells regulates their *I*-region-restricted helper activity in a primary antibody response (Fig. 5). This specificity might be mediated by allo-*I-A* and self-*I-A* receptors that could conceivably represent different molecules on distinct T cell subsets. From these studies and those of others on T helper cells obtained from chimeric mice (reviewed in 5, 8,

9, 57), it is evident that the phenotype of *H-2* restriction is not determined by the *H-2* genotype of T helper cells.

As an alternative hypothesis, allo-Ia and self-Ia receptor molecules may be functionally distinguishable yet structurally homologous, or even identical. It is possible that such an alloreactive T cell receptor can bind to allo-Ia with high affinity and to self-Ia with lower affinity; our studies (18, 30) on the alloreactive, Ia^-- and *I-A*-restricted component of allogeneic effect factor (AEF), a likely candidate for a T cell Ia alloantigen receptor, are compatible with this notion. Thus, it remains to determine whether the self-reactive $I\text{-}A^{k\ominus}$ donor T cells characterized here can also help B cells of a haplotype that differs from the host haplotype used. Several T cell clones that proliferate in response to both self-Ia plus antigen and allo-Ia in the absence of antigen have been identified (5–7, 58). It is not known whether allospecific T helper cells recognized allo-Ia antigens independently or allo-Ia antigens in association with other cell surface antigens. The frequency observed thus far for such heteroclitic T cell clones is ~5% of that observed for T cell clones that are only self-reactive. Further genetic and biochemical experimentation with GVHR-induced alloreactive T helper cell clones of either self-*I-A* and/or allo-*I-A* specificity is required to resolve the structural relationship between self-Ia and allo-Ia T cell receptors. It is anticipated that such studies, together with those on the *I-A* restricted helper component of AEF, will further clarify the biological significance of alloreactivity.

Finally, although alloactivated donor T cells collaborate with nonimmune B cells in an *I*-region-restricted in vitro primary response (Fig. 5), no *I*-region restriction is necessary for the interaction of these donor T cells with immune B cells in an in vitro secondary response (Fig. 6). These observations directly confirm those of Melchers et al. (59), who demonstrated that *I*-region-restricted immune responses occur with small, resting, antigen-unprimed B cells but not with large, dividing, antigen-primed and/or LPS-primed B cell blasts. Very similar findings have been obtained with the 68,000 mol wt, *I-A*-restricted component of AEF (18) and a 70,000 mol wt, antigen-nonspecific but *I-A*-restricted component of an SRBC-specific cloned T helper cell line (60); both of these T cell products are I-region restricted in their helper activity for nondividing unprimed B cells but not for dividing primed B cell blasts. Thus, once B cells have been stimulated to divide by either an antigenic or mitogenic stimulus, recognition of their surface Ia antigens by cooperating T cells and macrophages apparently is no longer required for effective interaction.

Summary

Graft vs. host response (GVHR)-activated donor T cells bind to stimulatory host cell-derived Ia antigens. Radioimmune cell-binding assays demonstrate that activated donor T cells acquire both host *I-A* and *I-E* alloantigens on their surface. Approximately threefold to fivefold less *I-E* products than *I-A* products are transferred. Immunoprecipitation and one-dimensional and two-dimensional gel electrophoresis analyses show that radioiodinated α and β polypeptide chains of both *I-A*- and *I-E*-encoded host Ia molecules may be transferred in an apparently structurally unaltered form from host cells to donor cells. Biosynthetic studies indicate that [^{35}S]methionine-labeled activated donor T cells do not synthesize Ia antigens of the donor haplotype. Functional analyses with fluorescence-activated cell sorter sorted donor T cell subpopulations show that donor T cells that bind host *I-A* antigens preferentially

cooperate with nonimmune host B cells. Donor T cells that do not bind detectable amounts of host *I-A* antigens preferentially help nonimmune donor B cells. By contrast, donor T cells that either bind or do not bind host *I-A* antigens display no *H-2*-restricted interaction and help both donor and host immune B cells. These data reveal that the Ia antigen-binding specificity of distinct functional subpopulations of alloactivated donor T cells regulates their *I*-region-restricted (self or allo) helper activity for nonimmune B cells but not immune B cells. Furthermore, they suggest that T cell-macrophage and T cell-B cell collaboration is mediated by a complementary anti-Ia:Ia receptor:ligand type of interaction in which the receptor of a T cell binds to the ligand of an antigen-presenting macrophage and/or B cell.

We especially thank Mr. Mark McCutcheon and Dr. Richard Miller for their valuable assistance and advice with the use of FACS, Ms. Kathy Louste for expert assistance with the maintenance and care of our mouse colony, and Ms. Donna Crawford and Ms. Mariana Arteaga for their cheerful assistance with the preparation of this manuscript.

Received for publication 8 June 1981.

References

1. Jerne, N. K. 1971. The somatic generation of immune recognition. *Eur. J. Immunol.* **1**:1.
2. Wigzell, H., and H. Binz. 1980. Lymphocyte receptors. *Prog. Immunol.* **IV**:94.
3. Benacerraf, B. 1978. A hypothesis to relate the specificity of T lymphocytes and the activity of I region-specific Ir genes in macrophages and B lymphocytes. *J. Immunol.* **120**:1809.
4. Pierce, S. K., N. R. Klinman, P. H. Maurer, and C. F. Merryman. 1980. Role of the major histocompatibility gene products in regulating the antibody response to dinitrophenylated poly(L-glu^{55}, L-ala^{35}, L-phe^{9})$_n$. *J. Exp. Med.* **152**:336.
5. Kappler, J. W., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible, *H-2*-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and *H-2* recognition. *J. Exp. Med.* **153**:1198.
6. Fathman, C. G., and M. Kimoto. 1981. Studies utilizing murine T cell clones: Ir genes, Ia antigens and MLR stimulating determinants. *Immunol. Rev.* **54**:57.
7. Sredni, B., and R. H. Schwartz. 1981. Antigen-specific, proliferating T lymphocyte clones. Methodology, specificity MHC restriction and alloreactivity. *Immunol. Rev.* **54**:187.
8. Moller, G., editor. 1978. Acquisition of the T cell repertoire. *Immunol. Rev.* **42**.
9. Von Boehmer, H. 1980. Expression of receptor diversity in T lymphocytes. *Prog. Immunol.* **IV**:113.
10. Moller, G. editor. 1978. Role of macrophages in immune responses. *Immunol. Rev.* **40**.
11. McMillan, M., J. M. Cecka, D. B. Murphy, H. O. McDevitt, and L. Hood. 1977. Structure of murine Ia antigens: partial NH_2-terminal amino acid sequences of products of the *I-E* and *I-C* subregion. *Proc. Natl. Acad. Sci. U.S.A.* **74**:5135.
12. Cook, R. G., E. S. Vitetta, J. W. Uhr, and J. D. Capra. 1980. The I-region of the murine major histocompatibility complex: genetics and structure. *In* Membranes, Receptors and the Immune Response. A Symposium Commemorating the 80th Anniversary of Erlich's Side Chain Theory. E. P. Cohen and H. Kohler, editors. Alan R. Liss, Inc., New York. 95.
13. Silver, J., and W. A. Russell. 1979. Structural polymorphism of *I-E* subregion antigens determined by a gene in the *H-2K* to *I-B* genetic interval. *Nature (Lond.)*. **279**:437.
14. Klein, J. 1980. Generation of diversity at MHC loci: implications for T cell receptor repertoires. *Prog. Immunol.* **IV**:239.
15. Prud-Homme, G. J., U. Sohn, and T. L. Delovitch. 1979. The role of H-2 and Ia antigens

in graft-versus-host reactions (GVHR). Presence of host alloantigens on donor cells after GVHR and suppression of GVHR with an anti-Ia antiserum against host Ia antigens. *J. Exp. Med.* **149:**137.

16. Delovitch, T. L., G. J. Prud'Homme, and U. Sohn. 1979. Ia phenotype and immunosuppression by anti-Ia of alloreactive donor T cells in a graft-versus host response. *In* The Biological Basis for Immunodeficiency Disease. E. Gelfand and H. M. Dosch, editors. Raven Press, New York. 111.
17. Delovitch, T. L., J. F. Harris, R. Battistella, and K. Kaufman. 1981. GVH host Ia antigens on activated donor T cells. *J. Supramol. Struct. Cell. Biochem. Suppl.* **5:**178.
18. Delovitch, T. L., and M. L. Phillips. 1981. The biological and biochemical basis of allogeneic effect factor (AEF) activity: relationship to T cell alloreactivity. *Immunobiology.* In press.
19. Delovitch, T. L., J. Biggin, and F. Y. Fung. 1978. 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. *J. Exp. Med.* **147:**1198.
20. Harris, J. F., and T. L. Delovitch. 1980. Derivation of a monoclonal antibody which detects and Ia antigen encoded by two complementing *I*-subregions. *J. Immunol.* **125:**2167.
21. Lerner, E. A., L. A. Matis, C. A. Janeway, Jr., P. P. Jones, R. H. Schwartz, and D. B. Murphy. 1980. Monoclonal antibody against an Ir gene product? *J. Exp. Med.* **152:**1085.
22. Hammerling, G. J., U. Hammerling, and Lemke, H. 1979. Isolation of twelve monoclonal antibodies against Ia and H-2 antigens. Serological characterization and reactivity with B and T lymphocytes. *Immunogenetics.* **8:**433.
23. Oi, V. T., P. P. Jones, J. W. Goding, L. A. Herzenberg, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. *In* Lymphocyte Hybridomas. Curr. Top. Microbiol. Immunol. F. Melchers, M. Potter, and N. L. Warner, editors. Springer-Verlag New York, Inc., New York. **81:**115.
24. Marshak-Rothstein, A., P. Fink, T. Gridley, D. H. Raulet, M. J. Bevan, and M. L. Gefter. 1979. Properties and applications of monoclonal antibodies directed against determinants of the Thy-1 locus. *J. Immunol.* **122:**2491.
25. Ey, P. L., S. J. Prouse, and C. R. Jenkin. 1978. Isolation of pure IgG_1, IgG_{2a}, and IgG_{2b} immunoglobulins from mouse serum using protein A Sepharose. *Immunochemistry.* **15:**429.
26. Wysocki, L. J., and V. L. Sato. 1979. Panning for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. U.S.A.* **75:**2844.
27. Delovitch, T. L., J. L. Press, and H. O. McDevitt. 1978. Expression of murine Ia antigens during embryonic development. *J. Immunol.* **120:**818.
28. Delovitch, T. L., and B. H. Barber. 1979. Evidence for two homologous, but nonidentical, Ia molecules determined by the *I-EC* subregion. *J. Exp. Med.* **150:**100.
29. Langone, J. J., M. D. P. Boyle, and T. Borsos. 1977. ^{125}I-protein A: applications to the quantitative determination of fluid phase and cell-bound IgG. *J. Immunol. Methods.* **18:**281.
30. Delovitch, T. L., J. Watson, R. Battistella, J. F. Harris, J. Shaw, and V. Paetkau. 1981. 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. *J. Exp. Med.* **153:**107.
31. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250:**4007.
32. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. *Eur. J. Biochem.* **56:**335.
33. Lalande, M. E., M. J. McCutcheon, and R. G. Miller. 1980. Quantitative studies on the precursors of cytotoxic lymphocytes. VI. Second signal requirements of specifically activated precursors isolated 12 h after stimulation. *J. Exp. Med.* **151:**12.
34. Delovitch, T. L., and H. O. McDevitt. 1977. In vitro analysis of allogeneic lymphocyte

interaction I. Characterization and cellular origin of an Ia-positive helper factor-allogeneic effect factor. *J. Exp. Med.* **146:**1019.

35. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology.* **14:**599.
36. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1978. Two-gene control of the expression of a murine Ia antigen. *J. Exp. Med.* **148:**925.
37. Jones, P. P., D. B. Murphy, D. Hewgill, and H. O. McDevitt. 1979. Detection of a common polypeptide in *I-A* and *I-E* subregion precipitates. *Mol. Immunol.* **16:**51.
38. Moosic, J. P., A. Nilson, G. J. Hammerling, and D. J. McKean. 1980. Biochemical characterization of the 31K polypeptide associated with *I-A* subregion Ia antigens. J. Immunol. **125:**1463.
39. Delovitch, T. L. 1981. Ia antigens: signals for lymphocyte communication. *Compr. Immunol.* In press.
40. Klein, J., and C. L. Chiang. 1976. Ability of H-2 regions to induce graft-versus-host disease. *J. Immunol.* **117:**736.
41. Clark, E. A., and W. H. Hildemann. 1977. Genetics of graft-versus-host reactions. I. Production of splenomegaly and mortality in mice disparate at *H-2I* subregions. *Immunogenetics.* **4:**281.
42. Clark, E. A., and W. H. Hildemann. 1977. Genetics of graft-versus-host reactions (GVHR). II. Interallelic effects and regulation of GVHR by antirecipient alloantibodies. *Immunogenetics.* **5:**309.
43. Cantrell, J. L., and W. H. Hildemann. 1972. Characteristics of disparate histocompatibility barriers in congenic strains of mice. I. Graft-versus-host reactions. *Transplantation (Baltimore).* **14:**761.
44. Schwartz, R. H., H. B. Dickler, D. H. Sachs, and B. D. Schwartz. 1976. Studies of Ia antigens on murine peritoneal macrophages. *Scand. J. Immunol.* **5:**731.
45. Schwartz, B. D., A. M. Kask, S. O. Sharrow, C. S. David, and R. H. Schwartz. 1977. Partial chemical characterization of Ia antigens derived from murine thymocytes. *Proc. Natl. Acad. Sci. U. S. A.* **74:**1195.
46. Delovitch, T. L., and H. O. McDevitt. 1975. Isolation and characterization of murine Ia antigens. *Immunogenetics.* **2:**39.
47. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1978. Identification of separate *I*-region products by two-dimensional electrophoresis. *In* Ir Genes and Ia Antigens. H. O. McDevitt, editor. Academic Press, Inc., New York. 203.
48. Charron, D. J., E. G. Engleman, C. J. Benike, and H. O. McDevitt. 1980. Ia antigens on alloreactive T cells in man detected by monoclonal antibodies. Evidence for synthesis of HLA-D/DR molecules of the responder type. *J. Exp. Med.* **152** Suppl.:127.
49. Yu, D. T. Y., J. M. McCune, S. M. Fu, R. J. Winchester, and H. G. Kunkel. 1980. Two types of Ia-positive T cells. Synthesis and exchange of Ia antigens. *J. Exp. Med.* **152** Suppl.: 89.
50. Nagy, Z., B. E. Elliott, and M. Nabholz. 1976. Specific binding of *K*- and *I*-region products of the *H-2* complex to activated thymus-derived (T) cells belonging to different Ly subclasses. *J. Exp. Med.* **144:**1545.
51. Elliott, B. E., Z. A. Nagy, B. J. Takacs, Y. Ben-Neriah, and D. Givol. 1980. Antigen-binding receptors on alloreactive T cells. Evidence of two closely-linked binding sites for allogeneic and self-MHC products. *Immunogenetics.* **11:**177.
52. Sharrow, S. O., B. J. Mathieson, and A. Singer. 1980. Cell surface appearance of host MHC phenotype on thymus cells from radiation bone marrow chimeras. *Fed. Proc.* **49:**945.
53. Paraskevas, F., and S. T. Lee. 1979. Macrophage-T cell interactions. IV. A new receptor on T cells: Ia antigens as receptors for Ig-antigen complexes which appear early after immunization. *Cell. Immunol.* **48:**31.

54. Nepom, J. T., B. Benacerraf, and R. N. Germain. 1981. Acquisition of syngeneic *I-A* determinants by T cells proliferating in response to poly ($Glu^{60}Ala^{30}Tyr^{10}$). *J. Immunol.* **127:** 888.
55. Puri, J., and P. Lonai. 1980. Mechanism of antigen binding by T cells. H-2 (I-A)-restricted binding of antigen plus Ia by helper cells. *Eur. J. Immunol.* **10:**273.
56. Rowley, D. A., H. Kohler, and J. D. Cowan. 1979. An immunologic network. *Contemp. Top. Immunobiol.* **7:**205.
57. Longo, D. L., and R. H. Schwartz. 1980. T-cell specificity for *H-2* and *Ir* gene phenotype correlated with the phenotype of thymic antigen-presenting cells. *Nature. (Lond.).* **287:**44.
58. Jones, B., and C. A. Janeway, Jr. 1981. Specificity and helper function of cloned T-cell lines. *J. Supramol. Struct. Cell. Biochem. Suppl.* **5:**139.
59. Melchers, F., J. Anderson, W. Lernhardt, and M. H. Schreier. 1980. Roles of surface-bound immunoglobulin molecules in regulating the replication and maturation to immunoglobulin secretion of B lymphocytes. *Immunol. Rev.* **52:**89.
60. Andersson, J., and F. Melchers. 1981. Antigen-and-Ia-specific and -unspecific molecules involved in T cell-dependent B cell stimulation. *Proc. Natl. Acad. Sci. U. S. A.* **78:**2497.