THE HISTOCOMPATIBILITY RESTRICTIONS ON MACROPHAGE T-HELPER CELL INTERACTION DETERMINE THE HISTOCOMPATIBILITY RESTRICTIONS ON T-HELPER CELL B-CELL INTERACTION

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The role of the major histocompatibility complex (MHC)[1] in the regulation of immunocompetent cell interactions has been intensively studied in several experimental systems including the interaction of antigen-pulsed macrophages and the primed T cell which proliferates on exposure to antigen in vitro (1, 2) and the interaction of the primed T-helper cell and the B cell in the generation of an antibody response (3). Both of these interactions appear to be controlled by the products of genes which map in the I-region of the MHC (2, 4, 5). The results of these experiments have suggested that the primed T cell is activated by carrier determinants of the nominal antigen in association with I-region associated (Ia) antigens on macrophages and the helper T cell, in turn, activates B cells which bear the same Ia antigens and determinants of the nominal antigen bound to immunoglobulin receptors on their surface. Although a large body of experimental evidence is consistent with this hypothesis, it has proven to be difficult to directly test experimentally because the majority of studies on macrophage T-cell interaction have used T-cell proliferation as a measure of T-cell activation, whereas the role of the macrophage as an antigen-presenting cell in the in vitro generation of a secondary antibody-forming cell response has proven to be difficult to define (6). In an attempt to directly correlate the results of studies on macrophage T-cell interaction with those on T- and B-cell interaction, we have examined the helper cell activity of primed guinea pig T cells which have been cultured in vitro for 7 days with antigen-pulsed macrophages under conditions which have been previously shown (7, 8) to be highly efficient in the antigen-specific selection of T cells which proliferate on re-exposure to antigen. We will demonstrate that these selected T cells function efficiently as T-helper cells only when mixed with syngeneic, but not allogeneic, hapten-primed B cells. Furthermore, when F_1 T cells are selected with antigen-pulsed parental macrophages, they will only cooperate with B cells of the same parental strain as the macrophages used in the selection culture. In addition, we will demonstrate in the well characterized poly-L-lysine system in

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[1] *Abbreviations used in the paper*: CFA, complete Freund's adjuvant; GL, a copolymer of L-glutamic acid and L-lysine; GPA, guinea pig albumin; Ia, I-region associated; Ir, immune response; LNL, column-purified lymph node lymphocytes; MHC, major histocompatibility complex; MLR, mixed leukocyte reaction; OVA, ovalbumin; PEC, peritoneal exudate cell; PEL, peritoneal exudate lymphocyte; PFC, plaque-forming cell; TNP, trinitrophenyl.

guinea pigs that the products of the histocompatibility-linked immune response (Ir) genes may also be functionally expressed in B lymphocytes.

Materials and Methods

Animals. Inbred strain 2, strain 13, and (2 × 13)F_1 guinea pigs were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Maryland.

Antigens. Picryl chloride was obtained from Polysciences, Inc., Warrington, Pa. A copolymer of L-glutamic acid (60%) and L-lysine (40%) (GL) with an average mol wt of 40,000 was purchased from the Pilot Chemical Division of the New England Nuclear Corp., Boston, Mass. Guinea pig albumin (GPA) and ovalbumin (OVA) were obtained from Miles Laboratories Inc., Miles Research Products, Elkhart, Ind. 2,4,6-trinitrophenyl (TNP)-GL, TNP-GPA, and TNP-OVA were prepared according to the method of Little and Eisen (9).

Immunization of Guinea Pigs. Animals to be used as a source of hapten-primed spleen cells were immunized in the footpads with 1 mg of picryl chloride in compelte Freund's adjuvant (CFA, containing 0.4 mg/ml *Mycobacterium tuberculosis* $H_{37}Ra$, Difco Laboratories, Detroit, Mich.). Carrier-primed T cells were obtained from animals immunized either with 100 μg of GL, TNP-GPA, or OVA in 0.4 ml of CFA, administered as 0.1 ml of emulsion intracutaneously into each footpad.

Preparation of Lymphocytes and Macrophages. After 5–10 wk, the animals were sacrificed and spleen, lymph node, and peritoneal exudate cells (PEC), induced by a 25-ml i.p. injection of sterile Marcol 52 (Humble Oil and Refining Co., Houston, Tex.), were harvested. Rayon wool adherence column purified peritoneal exudate lymphocytes (PEL) and lymph node lymphocytes (LNL) were prepared from immune animals and used as a source of helper T cells (10). Spleen cells were teased and then pressed through a no. 60 mesh wire screen. Macrophages were purified from the PEC population by adherence to plastic Petri dishes.

Technique of Brief Antigen Exposure. Unfractionated PEC or adherence-purified macrophages from nonimmune donors were pulsed with either 100 μg/ml OVA, TNP-GL, TNP-GPA, or 20 μg/ml TNP-OVA in the presence of 25 μg/ml Mitomycin C (Sigma Chemical Co., St. Louis, Mo.) for 1 h at 37°C and then washed four times to remove unbound antigen. Identical results were observed with PEC and purified macrophages and the results of these experiments have been pooled.

Positive Selection of Helper T Cells. LNL or PEL (6 × 10⁶) from immune animals were mixed with 1 × 10⁶ antigen-pulsed macrophages in a total volume of 2 ml RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 300 μg/ml L-glutamine, 50 μg/ml Gentamycin, (Schering Corp., Kenilworth, N. Y.), 5 μg/ml 5-fluorocytosine, 5 × 10⁻⁵ M 2-mercaptoethanol, and 5% heat-inactivated normal guinea pig serum. The cultures were incubated for 1 wk at 37°C in 5% CO_2 in air. On the 3rd day of culture the medium was decanted and replaced with 2 ml of fresh medium. In preliminary experiments it was noted that maximum T-helper cell activity was obtained after 1 wk of culture. At this time, 20–30% of the starting cell number was recovered and the recovered cells appeared to be 80–90% small lymphocytes and 10–20% macrophages by morphological criteria.

In Vitro Plaque-Forming Cell (PFC) Assay. The assay of the PFC response has been described in detail (11). In brief, 10 × 10⁶ spleen cells from animals primed to picryl chloride in CFA were mixed with 1 × 10⁶ viable cells obtained from the positive selection cultures in the presence of continuous antigen or antigen-pulsed macrophages in 1.0 ml of Eagle's Hanks' amino acid medium (Media Production Section, National Institutes of Health) in 35-mm culture dishes (BioQuest, BBL, & Falcon Products, Becton, Dickinson Co., Cockeysville, Md.) and rocked gently. The cultures were fed daily with nutritional cocktail. After 4 days of culture, anti-TNP PFC were assayed using a slide modification of the method of Jerne et al. (12). TNP-specific responses were determined by using TNP-substituted erythrocytes, prepared according to the method of Rittenberg and Pratt (13). Indirect PFC were assayed by incubating the slides for 1 h with a 1/100 dilution of rabbit anti-guinea pig immunoglobulin. Duplicate cultures were established for each variable and plated on a single slide. In general, the results of duplicate cultures varied by <15% and the results are expressed as a mean of indirect PFC of two culture dishes. Direct PFC were generally <10% of indirect PFC and are not reported.

TABLE I

Selection of Helper T Cells with Antigen-Pulsed Macrophages

	First Culture		Second Culture			
Group	PEL from animals primed to	T Cells Selected with macrophages pulsed with	B Cells TNP-primed spleen cells	Second Antigen 0.1 μg of	Indirect anti-TNP PFC/dish Exp.	
					I	II
1		DNP-GPA	+	TNP-GPA	1,045	995
2			+	TNP-OVA	430	50
3	DNP-GPA + OVA	OVA	+	TNP-GPA	280	15
4			+	TNP-OVA	1,090	840
5		DNP-GPA + OVA	+	TNP-GPA	855	815
6			+	TNP-OVA	1,050	630
7	—		+	TNP-GPA	265	35
8	—		+	TNP-OVA	145	30

PEL from strain 13 guinea pigs which had been primed with DNP-GPA and OVA were cocultured with antigen-pulsed macrophages for 7 days (first culture). Selected PEL (1 × 10⁶) were mixed with TNP-primed strain 13 spleen cells (10 × 10⁶) and the PFC response was assayed on day 4 of the second culture. The background response (groups 7 and 8) reflects the number of PFC produced when spleen cells alone were cultured in the presence of antigen.

Results

In Vitro Selection of T-Helper Cells with Antigen-Pulsed Macrophages. In contrast to the histocompatability requirements observed for the interaction of antigen-pulsed macrophages and the primed T cell which proliferates on exposure to antigen in vitro (1), our previous studies demonstrated that both syngeneic and allogeneic antigen-pulsed macrophages were equally efficient in antigen presentation for the in vitro generation of a secondary PFC response (11). However, we could not distinguish whether these observations reflected a true difference in the cellular interaction requirements for the two T-cell subpopulations, or alternatively, whether the activation of the helper T-cell antibody-forming cell system was much more sensitive to low antigen concentration and the apparent presentation of antigen by allogeneic macrophages was secondary to leakage of antigen with subsequent uptake and presentation by macrophages which had not been depleted from the responding T- and B-cell populations (carryover).

To more fully study the histocompatibility requirements for the interaction of antigen-pulsed macrophages and primed T-helper cells, we have used a modification of a procedure developed in this laboratory for the antigen-specific selection of T cells which proliferate in response to antigen stimulation in vitro (7, 8). PEL from strain 13 guinea pigs which had been immunized with TNP-GPA and OVA were cocultured for 7 days with syngeneic macrophages which had been pulsed with TNP-GPA, OVA, or a mixture of the two antigens. The specificity of the helper T-cell activity was then assayed in a second culture with syngeneic TNP-primed spleen cells. T cells which had been cultured with TNP-GPA pulsed macropahges in the first culture functioned more efficiently as T-helper cells in the second culture when challenged with TNP-GPA then when challenged with TNP-OVA (Table I, groups 1 and 2); in contrast, T

TABLE II

In Vitro Selection of Helper T Cells with Antigen-Pulsed Syngeneic or Allogeneic Macrophages

Group	First Culture		Second Culture			
	T Cells					
	OVA-primed LNL strain	Selected with OVA-pulsed macrophages strain	B Cells TNP-primed spleen cell strain	Second Ag 0.1 μg of TNP-OVA	Indirect anti-TNP PFC/dish Exp.	
					I	II
1	2	2	2	+	8,400	4,310
2	2	13	2	+	290	565
3	2	2 + 13	2	+	8,000	3,900
4	Group 1 + 2		2	+	8,500	4,850
5	13	13	13	+	4,400	3,210
6	13	2	13	+	150	590
7	13	13 + 2	13	+	3,260	1,815
8	Group 5 + 6		13	+	4,310	3,450
9	—		2	+	70	550
10	—		13	+	185	440

LNL from animals primed to OVA were cocultured with syngeneic or allogeneic OVA-pulsed macrophages for 7 days. Selected LNL (1×10^6) were mixed with TNP-primed spleen cells (10×10^6) and the PFC response assayed on day 4 of the second culture. In groups 4 and 8, selected LNL (1×10^6) from each group were mixed with 10×10^6 TNP-primed spleen cells. The background response (groups 9 and 10) reflects the number of PFC produced when spleen cells alone were cultured in the presence of antigen.

cells selected with TNP-OVA pulsed macrophages functioned more efficiently as T-helper cells when challenged with TNP-OVA than with TNP-GPA (Table I, groups 3 and 4). PEL which had been initially cultured with macrophages that had been simultaneously pulsed with TNP-GPA and OVA funcitoned equally well as T-helper cells when challenged with either TNP-GPA or TNP-OVA in the second culture. Identical results were obtained when column-purified LNL were used as a source of T cells in the selection culture (Table II). It should be noted that all of the T-helper cell activity generated in the selection culture system is secondary to in vivo priming as we have not been able to prime helper T cells in vitro under these circumstances.

Failure to Select Helper T Cells with Antigen-Pulsed Allogeneic Macrophages. Because of our previous observation that antigen-pulsed allogeneic macrophages could generate a secondary PFC response, we next evaluated whether T-helper cells could be selected with allogeneic antigen-pulsed macrophages. LNL selected with OVA-pulsed syngeneic macrophages functioned efficiently as T-helper cells when mixed with syngeneic TNP-primed spleen cells and challenged with TNP-OVA in the second culture (Table II, groups 1 and 5). T cells selected with allogeneic OVA-pulsed macrophages failed to exhibit helper-cell activity when mixed with syngeneic TNP-primed spleen cells and challenged with TNP-OVA in the second culture (Table II, groups 2 and 6). T cells selected with allogeneic OVA-pulsed macrophages also failed to exhibit helper activity when mixed with allogeneic TNP-primed spleen cells (results not shown). The failure to demonstrate T-helper cell activity in cell populations cultured with allogeneic antigen-pulsed macrophages could be secondary to the ongoing mixed leukocyte reaction (MLR) in such cultures or to the induction of T-suppressor cells.

TABLE III

Selected T Cells are Restricted in their Interaction with Primed B Cells

Group	First Culture		Second Culture			Indirect anti-TNP PFC/dish		
	OVA-primed T-cells strain	OVA-pulsed macro-phages strain	TNP-primed spleen cells strain	Second Ag 0.1 µg TNP-OVA	Normal spleen cells strain	Exp. I	Exp. II	Exp. III
1	2	2	2	+	—	4,310	8,400	5,550
2	2	2	13	+	—	740	570	775
3	2	2	13	+	2	820	715	900
4	2	2	2	+	13	5,000	9,100	5,300
5	13	13	13	+	—	3,210	4,400	2,490
6	13	13	2	+	—	920	200	500
7	13	13	2	+	13	1,390	265	825
8	13	13	13	+	2	3,360	4,205	2,090
9	2 / 13	2 / 13	2	+	—	4,750	—	5,900
10	2 / 13	2 / 13	13	+	—	3,350	—	2,510
11	—	—	2	+	—	550	70	700
12	—	—	13	+	—	440	185	585

LNL from animals primed to OVA were cultured with syngeneic OVA-pulsed macrophages for 7 days. Selected 1 × 10⁶ LNL were then mixed with either syngeneic or allogeneic TNP-primed 10 × 10⁶ spleen cells and the PFC response assayed on day 4 of the second culture. Some of the cultures were supplemented with 1 × 10⁶ spleen cells from unprimed animals. In groups 9 and 10, 1 × 10⁶ selected LNL from each strain were mixed with 10 × 10⁶ TNP-primed spleen cells. The background response (groups 11 and 12) reflects the number of PFC produced when spleen cells alone were cultured in the presence of antigen.

However, T cells cultured with a mixture of antigen-pulsed syngeneic and allogeneic macrophages were only slightly less effective than T-helper cells selected by culture with syngeneic macrophages (Table II, groups 3 and 7). Furthermore, when T cells selected with syngeneic antigen-pulsed macrophages and T cells selected with allogeneic antigen-pulsed macrophages were mixed together in the second culture, no decrease in the PFC response was observed (Table II, groups 4 and 8). It is thus unlikely that suppressor T cells were generated during culture with allogeneic macrophages.

Genetic Restriction in the Interaction Between Selected T Cells and Hapten-Primed B Cells. The results presented thus far demonstrate that primed antigen-specific T-helper cells can only be selected by culture with syngeneic antigen-pulsed macrophages. We next evaluated the capacity of the selected T-helper cells to interact with syngeneic or allogeneic hapten-primed B cells. OVA-primed LNL selected with OVA-pulsed syngeneic macrophages cooperated with syngeneic, but not allogeneic TNP-primed B cells (Table III, groups 1, 2, 5, and 6). Although we have only presented the results of experiments with a single concentration of helper T cells (1 × 10⁶/dish), which was determined to be optimal in preliminary experiments, cooperation between selected T cells and allogeneic B cells was not observed at concentrations ranging from 0.1 × 10⁶ to 2 × 10⁶/dish (results not shown).

It is possible that the failure of the selected T cells to cooperate with allogeneic B cells was secondary to a deficiency of antigen-presenting cells of the appropriate histocompatibility type in the second culture. However, the failure of the selected T cells to collaborate with allogeneic B cells could not be corrected by the addition to the culture of normal spleen cells syngeneic to the selected T cells as a source of antigen-presenting cells (Table III, groups 3 and 7); identical results were observed if purified peritoneal macrophages were used in place of normal spleen cells (results not shown). It thus appears that the failure to generate PFC in the mixture of selected T cells and allogeneic B cells is secondary to a restriction of T-B-cell collaboration in addition to a possible restriction in macrophage T-cell interaction during the selection culture.

One difficulty in the interpretation of these experimental results should be noted. In the absence of a reliable marker for guinea pig T cells, we have used unseparated spleen cells as a source of hapten-primed B cells. It is thus possible that a negative allogeneic effect may have been generated during the course of an MLR between the mature splenic T cells and the selected cells of the opposite haplotype which make up the helper T-cell population. We believe this explanation does not account for the failure of allogeneic T-B-cell collaboration because the addition of normal allogeneic spleen cells failed to inhibit the collaboration between selected T cells and syngeneic hapten primed B cells (Table III, groups 4 and 8). Furthermore, the admixture of selected T cells of both strains did not inhibit the collaboration of syngeneic T and B cells (Table III, groups 9 and 10).

Genetic Restriction on the Interaction of (2 × 13)F₁ T-Helper Cells and Parental B Cells. Previous studies from this laboratory using antigen-specific T-cell proliferation as a measure of T-cell activation have demonstrated two distinct populations of (2 × 13)F₁ T cells which are capable of interacting with antigen-associated with macropahges from one or the other parental strain (14, 15). To test the hypothesis that the population of (2 × 13)F₁ T cells capable of interacting with antigen associated with macrophages of one parental strain would also be restricted in its interaction to B cells of the same haplotype, F₁LNL were selected in vitro with OVA-pulsed strain 2, strain 13, or (2 × 13)F₁ macrophages. The selected T cells were then mixed with TNP-primed strain 2, strain 13, or (2 × 13)F₁ spleen cells and the resultant PFC response measured 4 days later.

F₁ T cells selected with F₁ OVA-pulsed macrophages cooperated with hapten-primed spleen cells of both parental strains (Table IV, groups 11 and 12). In contrast, F₁ T cells selected with OVA-pulsed strain 2 macrophages cooperated much more efficiently with strain 2 or (2 × 13)F₁ spleen cells than with strain 13 spleen cells (Table IV, groups 1, 3, and 5); similarly, F₁ T cells selected with OVA-pulsed strain 13 macrophages cooperated more efficiently with strain 13 or (2 × 13)F₁ spleen cells than strain 2 spleen cells (Table IV, groups 6, 8, and 10). The failure of the selected T cells to cooperate with spleen cells of the parental strain whose macrophages were not used in the selection step was secondary to a failure of T-B-cell cooperation because addition to the cultures of normal spleen cells syngeneic to the macrophages used in the selection step did not lead to a reconstitution of the response (Table IV, groups 4 and 9). No evidence for a negative allogeneic effect secondary to an MLR between mature splenic T cells and macrophages of the opposite haplotype which might be present in the selected F₁ T-cell population was seen. Thus, the addition to the second cultures of normal allogeneic spleen cells did not inhibit the interaction of

TABLE IV

Selection of (2 × 13)F₁ Helper T Cells with Antigen-Pulsed Parental Macrophages

Group	First Culture T Cells OVA-primed (2 × 13)F₁ LNL selected with OVA-pulsed macrophage strain	Second Culture					
		B Cells TNP-primed spleen cell strain	Normal spleen cells strain	Second Ag 0.1 µg of TNP-OVA	Indirect anti-TNP PFC/dish Exp.		
					I	II	III
1		2	—	+	5,650	2,840	6,800
2		2	13	+	—	—	6,900
3	2	13	—	+	780	670	580
4		13	2	+	1,565	1,385	845
5		(2 × 13)F₁	—	+	—	—	8,200
6		13	—	+	4,800	4,300	5,400
7		13	2	+	—	—	4,700
8	13	2	—	+	865	260	325
9		2	13	+	1,100	665	520
10		(2 × 13)F₁	—	+	—	—	9,600
11	(2 × 13)F₁	2	—	+	5,500	4,705	—
12		13	—	+	5,800	2,940	—
13	—	2	—	+	730	385	85
14		13	—	+	825	165	150

LNL from (2 × 13)F₁ guinea pigs which had been primed with OVA were cultured with OVA-pulsed strain 2, strain 13, or (2 × 13)F₁ macrophages for 7 days. Selected LNL (1 × 10⁶) were then mixed with TNP-primed strain 2, strain 13, or (2 × 13)F₁ spleen cells (10 × 10⁶) and the PFC response was assayed on day of the second culture. The background response (groups 13 and 14) reflects the number of PFC produced when spleen cells alone were cultured with antigen. Some of the cultures were supplemented with spleen cells from unprimed donors.

the selected T cells and the hapten-primed spleen cells syngeneic to the macrophages used in the selection cultures (Table IV, groups 2 and 7).

The results of these experiments are strongly in favor of the view that the same population of F₁ T cells which is capable of interacting with antigen-pulsed macrophages of one parental haplotype is also restricted to its interaction to B cells of the same parental strain. As the experiments described in Table IV were performed by the addition of continuous antigen to the second culture, it was of interest to repeat these sudies and directly examine the interaction of selected T-helper cells with antigen-pulsed macrophages. Surprisingly, although the selected (2 × 13)F₁ T cells were highly restricted in their interaction to B cells of the same parental strain as the macrophage used in the selection culture (Table IV, group 1, 5, 7, and 11), no restriction was observed in the interaction of antigen-pulsed macrophages and the selected F₁ T cells. Thus, (2 × 13)F₁ T cells, which had been selected with strain 2 macrophages, would only cooperate with strain 2 spleen cells but could be activated by either strain 2 or strain 13 antigen-pulsed macrophages (Table V, groups 1 and 2); similarly, (2 × 13)F₁ T cells, selected with strain 13 macrophages, cooperated only with strain 13 spleen cells, but could be activated by strain 2 or strain 13 antigen-pulsed macrophages. It is likely that the response generated by antigen-pulsed

TABLE V

Failure to Demonstrate a Restriction between Selected T-Helper Cells and Antigen-Pulsed Macrophages

Group	T Cells OVA-primed (2 × 13)F₁ LNL selected with OVA-pulsed macrophage strain	B Cells TNP-primed spleen cell strain	Second Antigen TNP-OVA pulsed-macro-phage strain	Indirect anti-TNP PFC/dish Exp. I	II
1		2	2	5,650	6,800
2		2	13	4,550	5,300
3	2	2	2 ΔT	3,325	—
4		2	13 ΔT	2,195	—
5		13	2	785	965
6		13	13	780	580
7		13	13	4,800	5,400
8		13	2	5,550	4,950
9	13	13	13 ΔT	3,050	—
10		13	2 ΔT	3,350	—
11		2	13	695	1,430
12		2	2	865	325
13		2	2	730	85
14	—	2	13	605	175
15		13	13	825	250
16		13	2	710	215

(2 × 13)F₁ LNL were cultured with OVA-pulsed parental macrophages for 7 days. Selected T cells (1 × 10⁶) were then mixed with hapten-primed parental spleen cells (10 × 10⁶) and antigen-pulsed normal or heat treated (ΔT) macrophages (2 × 10⁵); the PFC response was assayed on day 4 of the second culture. The background response (groups 13–16) reflects the PFC response of spleen cells alone in the presence of antigen-pulsed macrophages.

macrophages allogeneic to those used in the selection culture represents leakage of antigen with subsequent uptake by macrophages and/or B cells present in the spleen cell population because macrophages which had been killed by heat treatment also were partially capable of generating a secondary PFC response (Table V, group 3, 4, 9, and 10). However, it should be noted that in a large number of other experiments in which we varied either the number of macrophages added to the second cultures or the concentration of antigen used in the pulsing step, we could not establish the experimental conditions where antigen-pulsed macrophages syngeneic to those used in the selection culture activated T-helper cell activity and antigen-pulsed allogeneic or heat-treated macrophages failed to do so (results not shown). Thus, although F₁ T helper cells can be selected by culture with antigen-pulsed parental macrophages and thereby are restricted in their subsequent interaction with hapten-primed parental B cells, the actual role of the macrophage as an antigen-presenting cell in the activation of T-helper cell activity is not clear.

The Role of Ir Genes in T- and B-Cell Interaction. We have previously demonstrated that the antigen-induced proliferative response of primed (responder × nonre-sponder)F₁ T cells to antigens, the response to which is controlled by Ir genes, can only be activated by antigen associated with macrophages of the responder parent (4, 16). To determine if a similar restriction existed in the interaction of macrophages and T helper cells, we selected (2 × 13)F₁ T-helper cells from an animal primed to GL (an antigen the response to which is controlled by an Ir gene linked to the strain

TABLE VI

Failure to Select (Responder × Nonresponder)F_1 T-Helper Cells with Antigen-Pulsed Nonresponder Macrophages

| Group | First Culture | Second Culture | | | |
| | T Cells GL-primed (2 × 13)F_1 LNLs selected with GL-pulsed macrophage strain | B Cells TNP-immune spleen cell strain | Second Ag 0.1 μg of TNP-GL | Indirect anti-TNP PFC/dish Exp. | |
				I	II
1	(2 × 13)F_1		+	2,725	1,055
2	2		+	3,570	1,140
3	13	F_1	+	165	45
4	2 + 13		+	3,225	780
5	—		+	65	45

LNL from (2 × 13)F_1 guinea pigs primed to GL were cultured with GL-pulsed strain 2, strain 13, or (2 × 13)F_1 macrophages for 7 days. Selected T cells (1 × 10^6) were then mixed with TNP-primed (2 × 13)F_1 spleen cells and the PFC response assayed on day 4 of the second culture. The background response (group 5) reflects the number of PFC produced when spleen cells alone were cultured in the presence of antigen.

2 MHC) with (2 × 13)F_1, strain 2, or strain 13 GL-pulsed macrophages. Only culture with responder strain 2 or (2 × 13)F_1 GL-pulsed macrophages led to the selection of T-helper cells which cooperated with (2 × 13)F_1 TNP-primed spleen cells when challenged with TNP-GL in the second culture (Table VI, groups 1-3). Suppressor cells were not generated by culture of F_1 T cells with nonresponder strain 13 GL-pulsed macrophages, as F_1 T cells selected with a mixture of GL-pulsed strain 2 and 13 macrophages functioned as well as F_1 T cells selected only with GL-pulsed strain 2 macrophages (Table VI, group 4).

We next examined the genetic restrictions on the interaction of the primed F_1 T-helper cell with TNP-primed B cells of the responder and nonresponder parental strains. (2 × 13)F_1 T cells from animals primed with both GL and OVA were selected with either GL or OVA pulsed (2 × 13)F_1 macrophages. F_1 T cells selected with OVA-pulsed F_1 macrophages cooperated with TNP-primed B cells of either parent in the generation of a secondary anti-TNP response (Table VII, groups 4 and 5). However, F_1 T cells selected with GL-pulsed F_1 macrophages cooperated only with responder strain 2 TNP-primed B cells (Table VII, groups 1 and 2). The failure of the GL-selected F_1 T cells to cooperate with the nonresponder strain 13 B cell could not be corrected by the addition to the culture of responder strain 2 spleen cells as a source of antigen-presenting cells (Table VII, group 3).

Discussion

In this report we have evaluated the T-helper cell activity of primed guinea pig T lymphocytes which have been restimulated in vitro with antigen-pulsed macrophages. The recovered cells functioned efficiently as T-helper cells and were specific for the antigen with which the macrophages had been pulsed. Helper T cells could only be selected with syngeneic, but not allogeneic, antigen-pulsed macrophages and would then collaborate only with syngeneic, but not allogeneic, hapten-primed spleen cells.

Our previous studies using both positive and negative selection procedures demonstrated that the population of (2 × 13)F_1 T lymphocytes which can be activated to proliferate by antigen-pulsed macrophages of one parental strain is independent of

TABLE VII

Genetic Restriction Between F_1 T Cells and Nonresponder B Cells

| Group | First Culture | Second Culture | | | | | |
| | T Cells GL and OVA-primed (2 × 13)F_1 PEL selected with F_1 macrophages pulsed with | B Cells TNP-primed spleen cell strain | Second Ag 0.1 μg of | Normal spleen cell strain | Indirect anti-TNP PFC/dish Exp. | | |
					I	II	III
1		2		—	4,650	6,630	3,050
2	GL	13	TNP-GL	—	365	115	450
3		13		2	445	145	300
4	OVA	2	TNP-OVA	—	5,500	6,230	5,950
5		13		—	5,800	3,610	4,650
6	—	2	TNP-GL	—	65	165	500
7	—	2	TNP-OVA	—	730	—	350
8	—	13	TNP-GL	—	30	50	200
9	—	13	TNP-OVA	—	825	—	150

LNL from (2 × 13)F_1 guinea pigs which had been primed with OVA and GL were cultured with OVA or GL pulsed (2 × 13)F_1 macrophages for 7 days. Selected LNL (1 × 10^6) were then mixed with TNP-primed strain 2 or strain 13 spleen cells. TNP-GL or TNP-OVA were then added to the cultures and the PFC response assayed on day 4. The background response (groups 6–9) reflects the number of PFC produced when spleen cells alone were cultured with antigen.

the population of cells that can be activated by antigen-pulsed macrophages of the other parent (14, 15). In the present report we have extended these studies and demonstrated that the helper T cell can be selected by nominal antigen in association with Ia antigens on macrophages and is then capable of interacting only with B cells which bear the same nominal antigen and the same Ia antigens. Thus, (2 × 13)F_1 T cells primed to the carrier OVA and selected with OVA-pulsed F_1 macrophages cooperated with TNP-primed B cells of either parent when TNP-OVA was added to the cultures. In contrast, F_1 T cells selected with strain 2 OVA-pulsed macrophages cooperated much more efficiently with strain 2 B cells, but not strain 13, wheras F_1 T cells selected with OVA-pulsed strain 13 macrophages cooperated with strain 13 B cells, but not strain 2. The restrictions on T- B-cell collaboration were real and not simply due to the B-cell preparation being the only source of macrophages. Addition to the second cultures of macrophages or spleen cells syngeneic to those used in the selection step did not restore the capacity of the selected F_1 T cells to collaborate with B cells of the parent whose macrophages were not used in the selection culture. It thus appears that there are two populations of (2 × 13)F_1 T-helper cells; one population is selected by culture with strain 2 macrophages and is then able to collaborate only with strain 2 B cells, whereas a second population is selected by culture with strain 13 macrophages and then can only collaborate with strain 13 B cells. Although we have mapped the genes which control macrophage T-cell interaction to the I-region of the guinea pig MHC (4), we have not as yet formally demonstrated that the same genetic region regulates T- and B-cell interaction.

Our results on the histocompatibility restrictions between selected T cells and hapten-primed B cells are consistent with the observations of Katz et al. (3, 5) made

several years age that when carrier-primed T cells and hapten-primed B cells were adoptively transferred to sublethally irradiated recipients successful T-B interaction was only observed when the interacting populations shared the I-A subregion of the mouse H-2 complex. An approach similar to ours has been recently described by Swierkosz et al. (17) who isolated mouse antigen-specific helper T cells based on their ability to bind to antigen-pulsed monolayers. Thus, when F_1 helper T cells were isolated on antigen-pulsed macrophages bearing one of the parental H-2 haplotypes, they would collaborate only with parental B cells of the same H-2 type as the macrophages used in the binding step. Again, the failure of restricted F_1 T cells to cooperate with B cells of the H-2 type not used in the binding step could not be corrected by the addition to the cultures of peritoneal macrophages of the appropriate H-2 haplotype. Sprent (18, 19) has also recently shown that F_1 T cells primed to heterologous erythrocytes in one parental strain develop excellent helper activity for B cells of this parental strain, but gave minimal help for B cells of the opposite strain. The appropriate control experiments demonstrated that the restrictions on macrophage T-cell interaction were distinct from those on T- B-cell interaction. In contrast to these studies, McDougal and Cort (20) have shown that when F_1 T cells are primed in vitro they would preferentially collaborate with B cells of the same parental H-2 type as the macrophages used for priming, however, supplementing the cultures with macrophages histocompatible with those used for priming restored helper activity for B cells of the parental H-2 type opposite from that used in priming. A similar result has been reported by Feldmann and co-workers (21). Although these latter two studies dealt with restrictions of helper T cells primed in vitro, it is not as yet clear if the site of priming (in vivo vs. in vitro) is responsible for the different experimental results.

We do not believe that suppression is responsible for any of the restrictions on cell interaction that we have observed in spite of the fact that we did not remove mature T cells from our B-cell source in the second cultures. However, because of the observations of Swain and Dutton (22) that the addition of as few as 1% allogeneic T cells from a normal donor can suppress the secondary response of syngeneic T and B cells, we have included several control experiments to rule out suppressive effects. First, suppressor cells were not generated during the selection cultures because helper T cells could be efficiently selected with a mixture of antigen-pulsed syngeneic and allogeneic macrophages. Admixture of F_1 T cells selected with one parental macrophage with F_1 T cells selected with the opposite type did not inhibit the interaction of the selected T cells with B cells syngeneic to the macrophages used in the selection step. Second, the addition of as many as 10% allogeneic spleen cells or PEC failed to inhibit the interaction of syngeneic T cells and B cells. It is not clear why suppression has been observed in some experimental systems, but not others. One of the advantages of the guinea pig strains used in our studies is that they differ only in the I-region of the MHC (23). Some of the genetic studies of Swain and Dutton (24) suggested that relatively weak suppression was seen in combinations where only an I-region difference was present.

We have thus far assumed that the antigen-presenting cell used in the selection cultures is of the monocyte-macrophage lineage and hence, have used the term macrophage to describe the cell. In the guinea pig, antigen-specific T-cell activation as measured by proliferation is induced by a population of cells which are present in an oil-induced peritoneal exudate, can be purified by adherence to plastic or glass, are not of T-cell origin, and are probably not typical B lymphocytes in that guinea

pig lymph node cells which contain 30–40% B lymphocytes and only 2–5% macrophages are relatively poor activators of T-cell proliferation when pulsed with protein antigens (25). However, marked heterogeneity does exist in the guinea pig macrophage population in that only 15–25% of purified oil-induced guinea pig macrophages could be killed by treatment with anti-Ia serum and complement and the Ia-negative macrophages were markedly deficient in their ability to present protein antigens to immune T lymphocytes and to function as stimulator cells in the MLR (26). Although the presence of Ia antigens on macrophages correlates with their ability to mediate these immunologic funcitons, it is not clear whether the presence or absence of Ia antigens reflects a stage in monocyte-macrophage differentiation or whether the Ia-bearing cells are of a different cell lineage.

The role of the macrophage as an antigen-presenting cell in the activation of a secondary antibody response in vitro is far from clear. It should be noted, that, paradoxically, we were unable to show a histocompatibility restriction in the second cultures between the antigen-pulsed macrophage and the F_1 T-helper cells which had been selected with antigen-pulsed macrophages so as to interact with one or the other parental strain B cells. Thus, F_1 T cells which had been selected with strain 2 macrophages cooperated with only strain 2 B cells, but could be activated by antigen associated with either strain 2 or 13 macrophages. One trivial explanation for this result, which is supported by the experiments using heat-killed macrophages, is that antigen is transferred from the pulsed cells to macrophages or other cells present in the spleen cell population. We have not been able to evaluate this possibility because we have been unsuccessful in our attempts to prepare populations of guinea pig B lymphocytes which are depleted of macrophages. Two alternative possibilities for this experimental result should be considered. The first is that the differentiation of the mature B lymphocyte to an antibody-producing plasma cell cannot be induced by antigen which is confined to a macrophage. Thus, the B cell would require a constant, albeit low, concentration of antigen bound to its receptors for differentiation to take place. Consistent with this hypothesis are the observations of D. Sieckmann and W. E. Paul (personal communication) that the stimulation of tritiated thymidine uptake in mouse spleen cells by a goat anti-mouse IgM antiserum or by purified anti-μ antibodies can only be produced by the continuous presence of antiserum and not by brief pulse exposure of the B lymphocyte to the reagent. Second, as noted above, it does not appear that the guinea pig T-cell proliferative response can be activated by antigen-pulsed B lymphocytes. However, the activation of T-helper cell activity in the secondary antibody-forming cell response which probably does not require T-cell proliferation might be mediated by antigen bound to B lymphocytes. Indeed, one possible explanation for our failure and the failure of others (6) to observe a histocompatibility restriction between the antigen-pulsed macrophage and the primed helper T cell is that the most efficient pathway of T-helper cell activation is direct presentation to the helper T cell of antigen bound to the primed hapten-specific B cell with subsequent direct activation of B-cell differentiation in the immediate milieu of the helper T cell.

In our experiments with antigens the response to which is controlled by I-linked genes we demonstrated that primed (responder × nonresponder)F_1 T cells will only collaborate with B cells of the responder parent. The defect in this system appeared to be at the level of the B cell in that the addition to the cultures of antigen-presenting

cells (macrophages or nonimmune spleen cells) of the responder type did not restore the ability of F_1 T cells to collaborate with nonresponder B cells. These results are consistent with the experiments of Katz et al. (27) who demonstrated in an adoptive transfer system that (nonresponder $\times$ responder)F_1 T cells would only collaborate with high responder B cells when challenged with the 2,4-dinitrophenol-derivative of the terpolymer L-glutamic acid, L-lysine, and L-tyrosine. Similar results have been recently reported in an in vitro system by Kappler and Marrack (28). All these experimental results together with our earlier experiments on the failure of the nonresponder macrophage to activate (responder $\times$ nonresponder)F_1 T-cell proliferations are consistent with the hypothesis that the histocompatibility-linked Ir genes are functionally expressed in both macrophages and B lymphocytes, but not in T lymphocytes. However, the experimental data are also consistent with the view that Ir genes need only be expressed in macrophages. Thus, one might envisage that in the unprimed (nonresponder $\times$ responder)F_1 animal, clones of T cells exist that are capable of interacting with antigen associated with either responder or nonresponder Ia antigens. The critical step mediated by the products of the Ir genes (or the Ia antigens themselves) would be to create the linkage or association of antigen with Ia antigens. During the course of immunization such an association would occur in the F_1 macrophage and the clone of T cells capable of interacting with antigen and responder type Ia would be activated and expanded. This clone of T cells could then subsequently only cooperate with B cells that bear antigen and responder type Ia antigen, yet no requirement for Ir gene function to link or associate the nominal antigen with the Ia antigen need exist at the level of the B lymphocyte. The T lymphocyte once primed with the appropriate complex generated by the macrophage might recognize the Ia antigen and the nominal antigen separately on the B-cell surface.

Summary

To study the histocompatibility restriction between macrophages and helper T cells, carrier primed guinea pig T cells were positively selected in vitro with antigen-pulsed macrophages for 7 days and the selected T cells were then mixed with hapten-primed B cells and stimulated with antigen in a modified Mishell-Dutton system. Helper T cells could only be selected with syngeneic, but not allogeneic, antigen-pulsed macrophages and would then collaborate only with syngeneic, but not allogeneic, hapten-primed spleen cells. When F_1 T cells were selected with antigen-pulsed parental macrophages they would only collaborate with B cells of the same parental strain as the macrophages used in the selection culture. These results are strongly in support of the view that the primed T cell is activated by carrier determinants of the nominal antigen in association with Ia antigens on macrophages and the helper T cell, in turn, activates B cells which bear the same Ia antigens and determinants of the nominal antigen bound to immunoglobulin receptors on their surface. In addition, in experiments with antigens the response to which is controlled by I-linked genes, we demonstrated that primed (responder $\times$ nonresponder)F_1 T cells would only collaborate with B cells of the responder parent. The defect appeared to be at the level of the B cell in that the addition to the cultures of antigen-presenting cells of the responder type did not restore the ability of F_1 T cells to collaborate with nonresponder B cells.

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