Proc. Natl. Acad. Sci. USA
Vol. 78, No. 4, pp. 2497–2501, April 1981
Immunology

T cell-dependent activation of resting B cells: Requirement for both nonspecific unrestricted and antigen-specific Ia-restricted soluble factors

(helper T cells/T-cell receptor/Ia-restriction/helper factors)

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Communicated by Hilary Koprowski, December 11, 1980

ABSTRACT **Cloned murine helper T cells, restricted to the Ia^b antigens of the major histocompatibility locus and specific for horse erythrocytes as a foreign antigen, produce, in cooperation with antigen and histocompatible adherent cells, soluble factors that replace the helper T cells in their action on B cells. Three types of factors can be distinguished on the basis of molecular weight: proteins having apparent M_r 30,000 (p30) that act antigen- and Ia-nonspecifically as replication- and maturation-inducing factors and proteins having apparent M_r 55,000 (p55) and 125,000 (p125) that act on resting B cells in an Ia-specific, restricted fashion. Neither horse erythrocytes (a T-cell specific antigen) nor p55 and p125, alone or together, stimulate resting B cells to proliferation and maturation. Double occupancy by antigen *and* p55 or p125, however, renders Ia-compatible, but not Ia-incompatible, resting B cells susceptible to stimulation. The subsequent addition of p30 to these "excited" B cells then results in the proliferation and maturation of clones of horse erythrocyte-specific B cells. p55, but not p125, also cooperates with a "bystander" antigen, sheep erythrocytes, to excite sheep erythrocyte-specific resting B cells, which then replicate under the stimulatory action of p30. p30 do not bind antigen, nor do they bind anti-Ia or anti-immunoglobulin antibodies. p55 are bound by anti-heavy chain variable region antibodies, but not by anti-heavy or anti-light chain constant region antibodies or anti-Ia antibodies. p125 molecules bind horse but not sheep erythrocytes and are bound by anti-heavy chain variable region, but not by anti-heavy or light chain constant region or anti-Ia antibodies. p55 and p125 are likely to be soluble analogues of the antigen-specific, Ia-restricted T-cell receptors of the cloned helper T cells.**

From previous studies (1–3) done with cloned antigen-specific, Ia-restricted helper T cells, we concluded that three types of proteins on B cells regulate excitation from the resting state and stimulate proliferation and immunoglobulin secretion: (*i*) surface immunoglobulin, which binds antigens; (*ii*) surface Ia, which binds Ia-complementary structures ("anti-Ia"); and (*iii*) receptors for B-cell replication and maturation factors (BRMF), which bind BRMF (Fig. 1). It was found that Ia complementarity (anti-Ia) is supplied to B cells by cloned helper T cells and that the production of BRMF, which is dependent on helper T cells, Ia-histocompatible macrophages, and antigen, occurs in either helper T cells or macrophages. Our previous studies also suggested that BRMF activity can be found in media conditioned by T-cell help and therefore resides in soluble proteins (3). In this paper, we describe the separation and identification of proteins found in T-cell help-conditioned media that affect the interactions between T and B cells via Ia antigens and receptors for BRMF.

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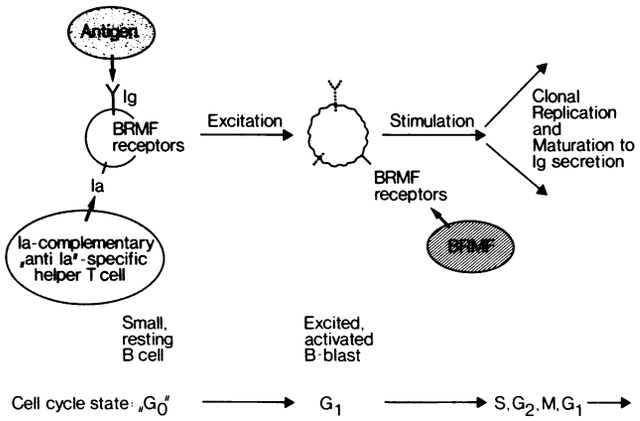

FIG. 1. Model of B-cell excitation and stimulation by T-cell help. Double occupancy on resting B cells of immunoglobulin, by antigen, and of Ia, by helper T cells, leads to functional excitation of receptors for BRMF. These receptors respond to BRMF in the excited, but not in the resting state, stimulating B cells to replicate.

MATERIALS AND METHODS

Animals. C57BL/6J/Fül, BALB/C/Fül, and C3H/Tif/Fül mice were obtained from the Institut für Biologisch-Medizinische Forschung A. G. (Füllinsdorf, Switzerland). B10.A(4R), B10.A(5R), and B10.A mice were obtained from Oxfordshire Laboratory Animal Colony (OLAC, 1976) (Oxon, England). B10.MBR mice were bred in our Institute, initiated from a breeding pair kindly provided by D. Sachs (National Institutes of Health).

Cell Cultures. Mouse spleen cells, prepared as described (4, 5), were cultured in Iscove's medium (6) containing transferrin, albumin, and soybean lipid as serum replacements, 2-mercaptoethanol (50 μM), and kanamycin (Bio-Cult, Irvine, Scotland). Spleen cell suspensions were enriched for small resting cells by velocity sedimentation (7, 8). Treatment of lymphocytes with the rat–mouse hybridoma J-1-j that produces monoclonal anti-thy 1.2 antibody (kindly given to us by J. Sprent, University of Pennsylvania, Philadelphia, PA) was carried out as described (2). Splenic B cells were activated in mass cultures at 3×10^5 cells per ml by various culture supernatants or by lipopolysaccharide (LPS), smooth form, obtained from

Abbreviations: HRBC, horse erythrocytes; SRBC, sheep erythrocytes; V_H, heavy chain variable region; BRMF, B-cell replication and maturation factors; LPS, lipopolysaccharide; TCGF, T-cell growth factor; PFC, plaque-forming cells; TRF, T-cell replacing factor; p30, p55, and p125, proteins having M_r 30,000, 55,000, and 125,000, respectively, that can replace helper T cells in their action on B cells.

Salmonella abortus equii, a gift from C. Galanos and O. Lüderitz, Max-Planck-Institut für Immunbiologie, Freiburg i. Br., Federal Republic of Germany (50 μg/ml). Activated B-cell blasts or nonreplicating, blast-like B cells were enriched by velocity sedimentation (7, 9).

Helper T cells specific for horse erythrocytes (HRBC) were obtained from lymph nodes of C57BL/6J/Fü1 mice inoculated subcutaneously at the base of the tail 7 days previously with 10^7 HRBC in Freund's complete adjuvant (10). They were cultured at concentrations between 5×10^4 and 4×10^5 cells per ml in serum-substituted medium (see above) in the presence of HRBC (2.5×10^6/ml) and 1×10^6 C57BL/6J/Fü1 spleen cells irradiated with 3300 rads (1 rad = 0.01 gray). Medium, cells, and antigen were replaced at weekly intervals, at which times the concentration of viable cells was readjusted to 5×10^4/ml. After 6–8 weeks, T-cell growth factor (TCGF), partially purified on Sephadex G-100 (11–13), was added to the growing T cells. Vigorous replication of the T cells resulted. The T cells were then alternately grown in antigen plus irradiated spleen cells (see above) for 7 days and in TCGF for 3 or 4 days. After 2 more months, the T cells were cloned by limiting dilution in medium containing TCGF, antigen, and irradiated spleen cells.

Production of Conditioned Media. Serum-substituted culture media were conditioned by incubation of 2.5×10^4 cloned helper T cells previously grown in TCGF for 3 or 4 days, HRBC at 2.5×10^6/ml, and irradiated C57BL/67/Fü1 spleen cells at 1×10^6/ml for 48 hr at 37°C in an incubator at 10% CO_2/90% air (3). The cells were then removed by centrifugation and the conditioned medium was filtered through a 0.45-μm Millipore filter. The medium could then be stored at 4°C for several weeks without appreciable loss of activity.

Concentration of Conditioned Media and Sephadex G-100 Gel Filtration. Conditioned media (typically 200–400 ml) were concentrated by precipitation with 80% saturated ammonium sulfate (ultrapure, Schwarz/Mann). The precipitate was collected by centrifugation (Sorvall, GSA head, 10,000 rpm for 30 min), and the resulting pellet was dissolved in 10 ml of 0.9% NaCl/0.01 M Hepes, pH 7.3 and dialyzed against the same buffer for 4 hr at 4°C. The precipitate that formed was removed by centrifugation, and the supernatant was applied to a Sephadex G-100 column (100 × 5 cm) equilibrated with 0.9% NaCl/0.01 M Hepes, pH 7.3. The column was eluted at 4°C with the same buffer; fractions (14–15 ml) were collected and sterilized by filtration through 0.45-μm Millipore filters. Fractions were tested in the B-cell assays at 5–10%.

Assays. LPS-activated B blasts were used at 1×10^5 cells per ml. Resting anti-thy 1.2-treated B cells were used at 3×10^5 cells per ml in all restimulation assays. Incorporation of [^{3}H]thymidine [1 μCi per 0.2 ml of culture (2 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels; Radiochemical Centre, Amersham, England)] for 2 hr at 37°C was carried out as described (3, 8). The number of cells in the culture secreting IgM was determined by the protein A-sheep erythrocyte (SRBC)-plaque assay (14). Protein A was from Pharmacia. HRBC- and SRBC-specific activation of resting B cells was done in the presence of SRBC or HRBC at 2.5×10^6/ml. For the detection of direct PFC, HRBC- or SRBC-specific assays were done at days 6 or 7 of culture as described (5).

Absorptions. Peak fractions (0.5 ml) corresponding to apparent M_r of 30,000, 55,000, and 125,000 (p30, p55, and p125, respectively; see Fig. 2), were incubated for 30 min at 4°C, then for 30 min at 37°C with 5×10^7 packed HRBC or SRBC or with 0.1 ml of packed Sepharose 4B coupled with the various antibodies. The supernatant fluids were then tested for activity on histocompatible resting B cells. Solutions (1–10 mg/ml) of specific antibodies purified by ammonium sulfate precipitation and DEAE-cellulose chromatography in 0.9% NaCl/0.01 M K phosphate, pH 7 were coupled to CNBr-activated Sepharose 4B (15). Specificities of the anti-immunoglobulin antibodies used (see Table 2) were those published previously (5). Anti-δ antibodies secreted by the mouse anti-mouse δ hybridoma H6-31 were a gift of T. Pearson and C. Milstein (Medical Research Council, Laboratory of Molecular Biology, Cambridge, England). Anti-heavy chain variable-region (V_H) antibody was a rabbit antiserum (16) kindly given to us by D. Givol (Weizmann Institute of Science, Rehovot, Israel).

RESULTS

T Cell-Replacing Activities in T-Cell Help-Conditioned Media. Cloned HRBC-specific helper T cells, HRBC, and irradiated spleen cells were incubated together for 48 hr. When the supernatant medium from such cultures (referred to here as T-cell help-conditioned medium) was added to LPS-activated B blasts of different Ia haplotypes, the B blasts were induced to proliferate further (Table 1). The polyclonal activator LPS had the same effect. This confirms earlier findings (1, 3) that restimulation of B blasts by T-cell help factors is polyclonal and Ia independent. It is therefore evident that T-cell help-conditioned media contain antigen- and Ia-unspecific factors that act like mitogens [i.e., BRMF (17)].

Whereas BRMF promote proliferation of already activated B blasts, their effect on resting B cells is to induce polyclonal, Ia-unrestricted maturation and IgM secretion without proliferation (3, 17). Hence, restimulation of proliferation in B blasts and stimulation of IgM secretion without proliferation in resting B cells were used as the basis for identification of molecules having BRMF activity.

The addition of antigens such as HRBC or SRBC to T-cell help-conditioned media does not change the response of activated B blasts. In contrast, an antigen-specific plaque-forming cell (PFC) response, which is a result of antigen-specific B-cell clones proliferating and maturing to immunoglobulin-PFC, is induced in small resting B cells (3).

A "bystander" antigen, SRBC, which is unrelated to the specificity of the cloned helper T cells (anti-HRBC) used to produce the conditioned media, induces clonal proliferation and immunoglobulin secretion in B cells specific for SRBC (2, 3). This antigen-specific clonal proliferation and maturation of resting HRBC- and SRBC-specific B cells only occurs when the B cells are histocompatible with the T-cell help. Tests with resting B cells from recombinant inbred strains (see Table 1) show that histocompatibility of helper T cells with Ia^b antigens directs the specificity of the interactions. Thus, we conclude that media conditioned by cloned T-cell help contain two activities: BRMF and another that acts Ia specifically on different states of B cells. We therefore attempted to separate these activities.

Separation of the BRMF from Ia-Specific T-Cell Help Factors by Gel Filtration on Sephadex G-100. Gel filtration on Sephadex G-100 of the factors contained in T-cell help-conditioned media gave a single peak (p30) having BRMF activity on proliferating B-cell blasts and on small resting B cells (Fig. 2A). It should be emphasized that all molecular weight determinations by Sephadex G-100 gel filtration (see also below) are only approximate. When either HRBC or SRBC were added as antigen, none of the fractions from the Sephadex G-100 column, including those with BRMF activity, induced antigen-specific clonal proliferation and maturation in resting B cells (see Table 1), a result in marked contrast to the findings obtained by using unseparated T-cell help-conditioned media. This suggests that the Ia-specific activities of the conditioned media, required for

Table 1. Effects of (bbb) T-cell help-conditioned media and of p30, p55, and p125 on histocompatible or incompatible LPS-activated B blasts and resting B cells

Addition	System*	B blasts (thymidine uptake), cpm per 10^5 cultured cells		Small resting B cells, PFC per 10^5 cultured cells					
				IgM-PFC		HRBC specific		SRBC specific	
		bbb	kkk	bbb	kkk	bbb	kkk	bbb	kkk
		Media†							
T-cell help-conditioned medium†	B cells alone	86,000	72,000	10,000	12,000	<20	<20	<20	<20
	B cells + HRBC	ND	ND	12,000	10,000	350	<20	<20	<20
	B cells + SRBC	ND	ND	15,000	15,000	<20	<20	240	<20
	B cells + HRBC + SRBC	92,000	77,000	14,000	16,000	400	<20	270	<20
None	B cells alone	1,000	1,000	100	150	<20	<20	<20	<20
	B cells + HRBC + SRBC	2,000	2,000	150	150	<20	<20	<20	<20
LPS at 50 μg/ml	B cells alone	110,000	85,000	80,000	85,000	220	100	120	100
	B cells + HRBC + SRBC	92,000	86,000	100,000	90,000	300	350	200	200
		Sephadex G-100 peaks‡							
p30	B cells alone	43,000	45,000	8,000	7,000	<20	<20		
	B cells + HRBC	46,000	40,000	5,000	6,000	<20	<20	ND	
p30 (1:10 dilution)	B cells + HRBC	1,500	1,000	100	100	<20	<20		
p125	B cells alone	1,500	500	100	150	<20	<20	ND	
	B cells + HRBC	1,000	1,000	150	150	<20	<20		
p55	B cells alone	1,000	1,500	100	50	<20	<20	ND	
	B cells + HRBC	500	1,000	100	50	<20	<20		
p30 + p125	B cells alone	46,000	52,000	9,500	7,500	<20	<20		
	B cells + HRBC	52,000	42,000	6,000	6,000	250	<20		
p30 + p125 ($1:10^2$ dilution)	B cells + HRBC	ND	ND	8,500	6,500	280	<20	ND	
p30 + p125 ($1:10^5$ dilution)	B cells + HRBC	ND	ND	8,000	7,000	30	<20		
p30 + p55	B cells alone	47,000	40,000	7,500	6,000	<20	<20		
	B cells + HRBC	44,000	38,000	6,000	6,000	240	<20		
p30 + p55 ($1:10^2$ dilution)	B cells + HRBC	ND	ND	8,000	7,000	240	<20	ND	
p30 + p55 ($1:10^5$ dilution)	B cells + HRBC	ND	ND	8,000	6,000	<20	<20		

B blasts: C57BL/6J/Fü1 (bbb) or C3H/Tif/BOM (kkk) spleen cells were activated for 48 hr with LPS at 50 μg/ml and purified by velocity sedimentation. Similar data were obtained with BALB/c (ddd), B10.A (kkd), B10.A(4R) (kkb), B10.A(5R) (bbd), and MBR (kbq) LPS-activated B blasts. Data for IgM-PFC in these cultures not shown as a measure of B-cell maturation were similar in numbers to those of thymidine cpm. B cells: C57BL/6J/Fü1 (bbb) or C3H/Tif/Fü1 (kkk) spleen cells were purified by velocity sedimentation (7) and treated with a monoclonal anti-Thy 1.2 antibody and complement as described in *Materials and Methods*. For the plaque assay, see *Materials and Methods*. The antigen-specific proliferation of $\approx 1/10^3$ small B cells is not sufficient for detection as an increase in thymidine uptake but is detectable through limiting-dilution analysis of HRBC- or SRBC-specific PFC clones (data not shown, see refs. 1 and 3). With T-cell help-conditioned medium and with no additions, uptake of [^{3}H]thymidine into small cells was $<10^3$ cpm per 10^5 cultured cells; LPS stimulated small B cells of both haplotypes to 3–4 × 10^4 cpm per 10^5 cultured cells, all measured at day 2 (see *Materials and Methods*). ND, not done.

* All HRBC and SRBC at 2.5 × 10^6/ml.

† 50% in culture.

‡ At 10% in culture.

induction of resting B cells, were either destroyed or separated from the BRMF activity during the process of concentration and gel filtration.

Detection of Ia-Specific T-Cell Help Factors on Sephadex G-100. If the activities required for induction of resting B cells are not destroyed but rather only separated from BRMF activity, it should be possible to find them under the condition that, in addition to the antigen (HRBC or SRBC), BRMF (p30) be provided in the resting B-cell cultures (see Fig. 1). When this was done, two peaks of activity inducing antigen-specific PFC responses were detected (p55 and p125) (see Fig. 2*B*). These peaks were detected only when histocompatible resting B cells were used in the assay (see Table 1). The activity of p55 and p125 was, therefore, Ia specific and Ia restricting. When the helper T cell-specific antigen, HRBC, was replaced in the assay by an antigen, SRBC, unrelated to the helper-cell specificity, activity was detectable only with p55 and only with resting B cells of Ia-compatible haptotypes. BRMF diluted 1:10 was inactive with either activated B blasts or resting B cells, whereas the Ia-specific activities of p55 and p125 could be diluted at least 1:1000 before reduction of activity (in the presence of a constant high amount of BRMF) became apparent.

In conclusion, Sephadex G-100 gel filtration separated the two activities into three peaks: the antigen- and Ia-nonspecific activity of BRMF as p30 and the Ia-specific activity as p55 and p125. Only p55 were active with a T-cell help-unrelated antigen. The Ia-specific proteins alone did not stimulate resting B cells to replicate and mature to clones of immunoglobulin-secreting PFC.

Absorption Studies. Neither of the antigens nor any of the immunoglobulin- and Ia-specific immunoabsorbents absorbed the BRMF activity (p30) (Table 2). HRBC, but not SRBC, absorbed the Ia-specific, B cell-restricting p125 but not the Ia-specific p55. We therefore conclude that p125 can bind specific antigen—i.e., that it is "anti-antigen" as well as "anti-Ia." As shown by the functional test with resting B cells, p55 is only anti-Ia. Neither of the Ia-specific proteins contains any of the detectable constant region determinants of light or heavy chains that are common to IgM, IgD, IgG, or IgA or any Ia antigenic determinants. Both p55 and p125 may contain V_H-like struc-

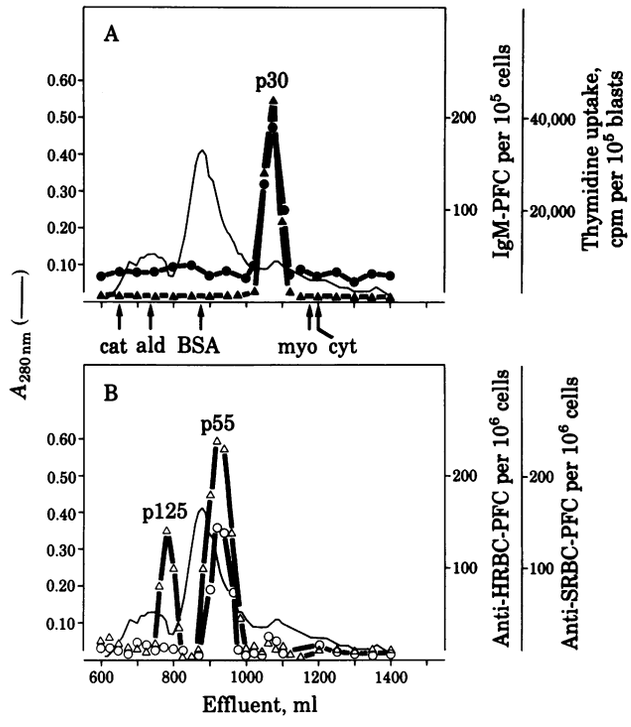

FIG. 2. Gel filtration on Sephadex G-100 of factors contained in media conditioned by T-cell help. (*A*) BRMF activity as assayed by restimulation of proliferation of B blasts on day 2 (●) and by stimulation of IgM-secreting cells without proliferation of resting B cells on day 4 (▲) of culture. M_r standards: cat, catalase (240,000); ald, rabbit aldolase (160,000); BSA, bovine serum albumin (67,000); myo, horse myoglobin (17,800); cyt, cytochrome *c* (12,400), all from Serva (Heidelberg, Federal Republic of Germany). (*B*) Ia-restricting activities of proteins that induce clonal proliferation and maturation in histocompatible (bbb) resting B cells in the presence of BRMF (p30) and either HRBC (△) or SRBC (○) as antigen. PFC assays were performed on day 7 of culture.

tures, if we assume that the rabbit anti-V_H antiserum recognizes only V_H and nothing else.

DISCUSSION

The action of BRMF on B cells is similar to that of TCGF on T cells. Both factors stimulate activated, but not resting, cells to proliferation; both act polyclonally; and both act in an Ia-unrestricted fashion. Both factors have the same approximate molecular weights—30,000 in the mouse and 15,000–18,000 in the rat (ref. 18; unpublished observations). Are BRMF and TCGF in fact the same protein acting on different cellular targets? A number of observations suggest that this is not the case.

Table 2. Absorption of activities of the three types of molecules from T-cell help-conditioned media separated on Sephadex G-100

	PFC formed, no. per 10^5 cultured resting cells				
		p55		p125	
Absorbant	p30 (IgM-PFC)	HRBC specific	SRBC specific	HRBC specific	SRBC specific
None	5500	270	165	150	<20
HRBC	6500	160	150	≤20	<20
SRBC	5000	280	165	140	<20
Anti-μ	7500	250	170	155	<20
Anti-δ	7000	180	150	135	<20
Anti-κ	6000	230	150	140	<20
Anti-λ	5000	270	140	100	<20
Anti-γ_1 + γ_2	7000	240	180	120	<20
Anti-α	6000	260	130	130	<20
Anti-V_H	6500	≤20	≤20	≤20	<20
Anti-Ia^b	6000	200	150	120	<20

SRBC or HRBC at 10^8 per 0.1 ml of Sephadex G-100 peak fraction (see Fig. 1) and antibodies coupled to Sepharose 4B; see *Materials and Methods*. Assays for IgM-PFC were at day 4 and those for HRBC- and SRBC-specific PFC were on day 7 of culture.

First, BRMF isolated as p30 on Sephadex G-100 from conditioned media of cloned, HRBC-specific, Ia-restricted T-cell help have very little, if any, restimulatory activity for killer T cells that depend on the presence of TCGF for clonal expression (18, 19). Second, BRMF can be obtained from media conditioned with allo-Ia-reactive T-cell lines in the presence of allo-Ia antigen-containing adherent cells and, again, such BRMF preparations have little restimulatory activity for clonal replication of murine killer T cells. Third, BRMF activity is detectable in media conditioned by concanavalin A-activated mouse or rat spleen cells (unpublished results), which are known to contain TCGF activity as well (18). However, when murine killer T cells are grown in such media, TCGF activity is depleted, while BRMF activity, if anything, is enhanced (unpublished observations). All of these observations support the idea that TCGF and BRMF are separate proteins. BRMF, like TCGF, apparently acts across species barriers.

It has been claimed that proliferation and maturation to immunoglobulin secretion are stimulated by separate proteins that act on B cells through separate receptors. The Ia-unrestricted, polyclonally acting maturation factor called TRF (20) has M_r ≈30,000. Recent evidence suggests that TRF and TCGF are different proteins (21). Our BRMF shares size and maturation-inducing activity with TRF. It remains, however, to be investigated whether or not TRF is a component of or the same protein as BRMF.

B-cell stimulation is antigen-specific and Ia-restricted. A resting B cell, in addition to antigen and BRMF (p30), therefore, has to bind Ia-compatible anti-Ia structures. We show in this paper that such anti-Ia structures, p55 and p125, are provided by helper T cells and can be isolated from conditioned media. This, however, does not necessarily imply that T–B collaboration *in vivo* is not effected by cell–cell contact. We also expect the same proteins to be active in helper T cell–macrophage interactions and, in fact, to be part of the T-cell receptor. This would mean that the same proteins, p55 and p125, are receptors for helper T cells and stimulating ligands for B cells and macrophages. The effects of p55 and p125 clarify the process of B-cell stimulation: binding of these anti-Ia proteins to resting B cells alone or together with antigen does not suffice to stimulate clonal replication. Together with antigen, however, but not alone, p55 and p125 render resting B cells susceptible to action by BRMF (p30), which then results in antigen-specific clonal replication and immunoglobulin secretion. p55 and p125 apparently do not carry the constant region structures of IgM, IgD, IgG, or IgA but crossreact with V_H-specific antisera. While it is tempting to speculate that they recognize Ia via V_H structures, we are aware of the possibility that the V_H-specific antisera may contain other specificities.

p125 binds antigen HRBC, for which the cloned helper T cells are specific. This suggests that p125 may be anti-Ia as well as anti-antigen; again, it is tempting to speculate that both activities may be effected by V_H structures. Because Ia is structurally different from HRBC, the two binding activities should have different binding structures.

Our finding that, although they are Ia-complementary and function with HRBC, p125 does not excite SRBC-specific resting B cells (see Table 1) while p55 excite both SRBC- and HRBC-specific resting B cells, may offer insight into the mechanism of the interactions of T-cell receptors with antigen and

with Ia determinants. The antigen-binding properties of p125 may dominate the Ia-recognizing properties of the same protein. Structurally, this may mean that the Ia-binding structures are only exposed when the antigen-binding structures already have their antigen bound, and this may be the way in which the T-cell receptor functions on an intact helper T cell.

A comparison of our findings with other information about T-cell factors and receptors (22) is outside the scope of this discussion. In addition, long-term cultured or cloned T-cell lines that have helper function have only recently been established.

It has recently been shown that several allo-Ia-reactive T-cell lines produce allogeneic effect factor (23–25) and that this factor acts in an Ia-specific restricting fashion, stimulating only those B cells that carry the allo-Ia-antigen for which the T cells are specific (25). Although the assays did not distinguish between excitation and stimulation of resting B cells, it was found that the Ia-specific, B cell-restricting proteins had M_r 50,000–70,000 and that the Ia-nonspecific proteins had M_r 30,000–35,000. These are remarkably similar to the proteins of our cloned helper cells (p30 and p55) with respect to both function and approximate M_r. Allogeneic effect factor apparently does not contain functionally active proteins of M_r higher than 70,000. This suggests that alloreactive helper T cells may have only a single receptor for alloantigen. It is also possible that higher M_r proteins are functionally inactive with allo-Ia-containing B cells, just as p125 is inactive with the bystander antigen SRBC (see Fig. 1 and Table 1).

Inasmuch as B-cell regulation is effected by a complex of proteins and a hierarchy of interactions with their ligands involving immunoglobulin, Ia, and BRMF receptors, T cells should also be regulated by receptors for TCGF, Ia determinants, and immunoglobulin-like receptors (which contain anti-antigen and anti-Ia binding sites). These immunoglobulin-like receptors on T cells appear to violate the rule that one lymphocyte makes one antibody. One T cell may make one V_H containing structure for anti-antigen and one V_H containing structure for anti-Ia, although possibly as a dimer in p125. This finding could lead to reconsideration of the same rule for the case of B cells and present a question as to whether or not V_H and V_L structures of one antibody on one B cell ever have separate specificities for antigen.

Our assays with resting B cells should be of use in the search for antigen-specific, Ia-specific T-cell receptor molecules produced by cellular sources such as T-cell tumors or synthesized in cell-free systems from DNA or RNA templates.

The able technical assistance of Ms. Lotta Haraldson and Ms. Denise Richerich is gratefully acknowledged. We thank Dr. J. Chiller for showing us how to activate helper T cells by the tail immunization procedure. For help with the manuscript, we thank Dr. H. Koprowski, Dr. N. Iscove, and Mrs. M. Courant. J.A. was supported by the Swedish Medical Research Council. The Basel Institute for Immunology was founded and is supported by F. Hoffmann–La Roche and Company, Limited, Basel, Switzerland.

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