

ANTIGEN-SPECIFIC, MHC NONRESTRICTED T HELPER
CELL-INDUCED B CELL ACTIVATION

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The isolation and characterization of structural proteins and genes that encode the α and β chains of the T cell antigen receptor have rapidly advanced our understanding of the molecular biology of these important molecules (1). However, the central issue of T cell receptor function, how T cells are constrained to recognize antigen only in conjunction with self MHC determinants remains unresolved (2).

A novel approach to this question was suggested by our recent observation (3) that direct (cognate) interaction between cloned allospecific human T helper (Th) cells and allogeneic B cells leads to the rapid expression of a B cell specific activation antigen, BLAST-2 (4, 5). Presumably, BLAST-2 expression is triggered by specific binding of the Th cell antigen receptor with DR antigens on the B cell surface. Of interest, we frequently observed low-intensity BLAST-2 expression on a small fraction of allogeneic B cells that had been cocultured with irrelevant Th cells, i.e., Th cells specific for DR antigens that the B cells do not express. This was surprising, as activation of these allospecific Th cells, assessed either by proliferation or helper activity for B cell differentiation, is absolutely MHC restricted (6, 7). These results suggest that the avidity of interaction between the Th cell antigen receptor and the B cell surface DR molecule required for BLAST-2 induction may be less than that required for Th cell activation. If so, Th-induced BLAST-2 expression may provide a sensitive functional assay for investigating the antigenic structure recognized by the T cell receptor. To this end, we have examined the induction of BLAST-2 by a cloned, IL-2-dependent, hapten-altered self-reactive human Th cell (8). These cells are uniquely suited to the experimental question as they have a dual specificity for both hapten and a polymorphic epitope expressed on self class II MHC molecules. Moreover, surface proteins on all B cells can be modified by haptens to serve as potential targets of a direct (cognate) Th cell interaction, and this interaction detected using the BLAST-2 expression assay. Our results demonstrate that cloned TNP-specific, MHC-restricted Th cells induce BLAST-2 expression on chemically modified B cells in a hapten-specific but MHC-nonrestricted manner. These

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results speak strongly against a T cell receptor that recognizes a "neoantigen" comprised of nominal antigen and polymorphic epitopes of MHC molecules.

Materials and Methods

Preparation of Responder B Cell Population. Adherent cell-depleted non-T cells, used as the responder B cell populations, were isolated from human peripheral blood or tonsils as previously described.

T Cell Clones. E-11 is an IL-2-dependent human Th clone specific for TNP presented by cells that express a polymorphic class II MHC epitope shared by DR8 and some DR5 molecules (8). Alloreactive Th clones A-57 and 33 are specific for DR2 and DR1, respectively (6, 7).

BLAST-2 Induction Assay. The induction of BLAST-2 by cloned Th cells has been described previously (3). Briefly, 10^6 B cells were incubated, in final medium alone or in the presence of 0.25×10^6 cloned Th cells at 37°C , 5% CO_2 for 16 h, and then assessed for BLAST-2 expression by indirect immunofluorescence staining using the EBVCS₂ mouse mAb (generously donated by Drs. Bill Sugden and Stan Metzenberg, McArdle Laboratory for Cancer Research, Madison, WI). The percentage of positively staining cells was determined on a cytofluorograph, gating on the lymphocyte population. Results are expressed as the percent of B1-bearing cells (Coulter Immunology, Hialeah, FL) positive for BLAST-2 after subtraction of background fluorescence. In some studies, as a positive control for biotinylation, an aliquot of the relevant B cell populations were stained with phycoerythrin-conjugated avidin (Tago Inc.).

E-11 Proliferative Assay. As previously described (8), E-11 cells (10^4) were cultured in triplicate with final medium alone or with 10^5 x-irradiated stimulator cells. After 24 h, cultures were pulsed with 0.2 μCi of [³H]TdR (5 $\mu\text{Ci}/\text{ml}$; New England Nuclear, Boston, MA). Incorporation of radioactivity was measured by liquid scintillation counting, and data expressed as mean cpm \pm SEM.

Plaque-forming Cell Assay. 10^6 responder B cells were cultured with final medium alone, 0.25×10^6 E-11 cells, or, as a positive control, 0.25×10^6 autologous T cells and 10 $\mu\text{g}/\text{ml}$ of PWM (Gibco Laboratories). After 6 d, each culture was assayed in triplicate for antibody-forming cells (AFC), using the reverse hemolytic plaque assay as previously described (6).

Chemical Modification of Cells. B cell populations were TNP modified as previously described (8). B cells to be biotinylated were suspended in bicarbonate-buffered saline (pH 8) containing 0.5 mg/ml of *N*-hydroxy succinimidobiotin (NHSB) (Sigma Chemical Co., St. Louis, MO) for 1 h at room temperature, then washed extensively.

Results

In the studies reported here, we used a well-characterized hapten-altered self-reactive human Th clone, termed E-11, which recognizes TNP in association with a polymorphic epitope expressed on DR8 and some DR5 molecules (8). Thus, E-11 cells were cocultured with unmodified or TNP-modified B cells (B_{TNP}) obtained from a panel of allogeneic donors of known HLA-DR haplotype, and after 16 h the B cells were analyzed for BLAST-2 expression by indirect immunofluorescence staining. The results of several representative experiments are presented in Table I. As expected, TNP-modified, DR5⁺ B cells, cocultured with E-11, are induced to express BLAST-2. Of greater interest, all TNP-modified B cells, regardless of their DR haplotype, express BLAST-2 after coculture with E-11. In contrast, E-11 does not trigger BLAST-2 expression on unmodified B cells, including B cells bearing the DR5 determinant, which restricts E-11 cell function. To rule out that TNP modification renders B cells nonspecifically responsive to Th cell influence, allospecific Th clones were

TABLE I
*Clone E-11-induced BLAST-2 Expression on TNP-modified B Cells
 Is MHC Nonrestricted*

B cell donor DR type	Responder B cells	% of BLAST-2 ⁺ B cells after culture with:		
		Medium	Clone E-11	Allospecific Clone
4, 6	B	2	1	ND
	B _{TNP}	1	34	ND
1, 5	B	2	7	ND
	B _{TNP}	2	44	ND
2, 6	B	1	3	4 (clone 33)*
	B _{TNP}	1	54	7 (clone 33)
3, 4	B	1	2	ND
	B _{TNP}	1	44	ND
2,5	B	1	1	ND
	B _{TNP}	1	39	ND
2, —	B	9	10	49 (clone A-57) [‡]
	B _{TNP}	9	37	42 (clone A-57)

Unmodified or TNP-modified B cells, obtained from a panel of donors of known DR haplotype, were cultured in medium alone or medium containing cloned Th cells as described above. After 16 h of culture, B cells were assayed for BLAST-2 expression by indirect immunofluorescence staining.

* Clone 33 (DR1 specific) does not recognize the DR antigens expressed by the responder B cell population in this study.

[‡] Clone A-57 (DR2 specific) recognizes and proliferates in response to the DR antigen expressed on this responder B cell population.

included as controls. Clearly (Table I), TNP-modified B cells are not triggered to express BLAST-2 by coculture with irrelevant allospecific Th clones. Moreover, unmodified and TNP-modified B cells appear to respond similarly to interaction with appropriate allospecific Th clones (Table I and Fig. 1). Finally, although E-11 induces BLAST-2 on TNP-modified B cells regardless of DR type, activation of E-11 remains hapten specific and MHC restricted. Thus, E-11 proliferates selectively to TNP-modified DR5⁺ cells and only TNP-modified DR5⁺ B cells are induced to differentiate into AFC by coculture with E-11 (Table II).

To prove hapten (TNP) specificity of BLAST-2 induction by E-11, B cells were modified with a second, noncrossreactive chemical, NHSB. This reagent was chosen because it covalently links biotin to amino groups (9) in a manner similar to trinitrobenzene sulfonic acid, and can be detected on the B cell surface by using avidin coupled to a photochrome. The results of these studies (Fig. 1) make three points. First, NHSB treatment of B cells results in effective conjugation of biotin to the B cell surface. Second, NHSB treatment does not inhibit BLAST-2 expression triggered by an allospecific Th clone (A-57). Third, and most important, while A-57 induces BLAST-2 on all three B cell populations, unmodified, B_{TNP}, and biotin-modified B cells, E-11 selectively triggers high-intensity BLAST-2 expression only on TNP-modified cells. Taken together, our data suggest that although Th cell activation requires interaction with antigen and restricting epitopes on class II molecules, Th cells can recognize nominal

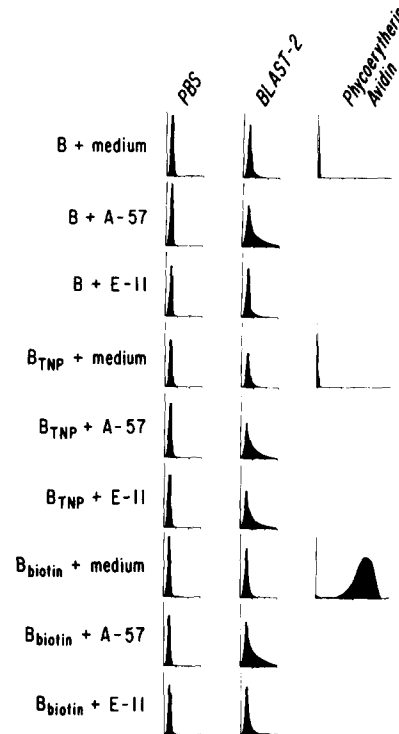


FIGURE 1. E-11 triggers BLAST-2 expression in an antigen (TNP)-specific manner. 10^6 unmodified, TNP-modified, or biotin-modified B cells were cocultured with final medium alone, 0.25×10^6 E-11 cells, or 0.25×10^6 A-57 cells. After 16 h of culture, expression of BLAST-2 was assayed by immunofluorescence staining. As a positive control for biotinylation, an aliquot of each B cell population was also stained with phycoerythrin-conjugated avidin. The percentage of clone-induced BLAST-2 expression was calculated by subtracting background BLAST-2 expressed by B cells cultured with medium alone from that expressed by B cultured with the Th clones. These results are as follows: B + A-57, 38%; B + E-11, 2%; B_{TNP} + A-57, 32%; B_{TNP} + E-11, 28%; B_{biotin} + A-57, 40%; B_{biotin} + E-11, 4%.

antigen alone sufficiently well to deliver an antigen-specific but MHC-unrestricted activation signal to B cells.

Discussion

These studies have implications for the functional properties of the T cell antigen receptor. Most clearly, our findings are not compatible with the idea of a receptor that recognizes a neoantigen created by the association of nominal antigen (hapten) and polymorphic class II MHC determinants, because E-11 induces BLAST-2 on all TNP-modified B cells regardless of their DR haplotype. In contrast, E-11 does not appear to recognize class II MHC determinants alone, as coculture of these helper cells with unmodified B cells bearing the relevant restricting class II MHC antigen (DR5) does not lead to BLAST-2 expression. This result may indicate that the portion of the T cell receptor that interacts with the MHC class II antigen is not capable of inducing BLAST-2 expression. This interpretation is not likely, in view of our studies (3) demonstrating MHC class II-specific BLAST-2 induction by allospecific Th cells. An alternative

TABLE II
Activation of E-11 is Antigen Specific and MHC Restricted

Description of cell culture	B cell DR type	[³ H]TdR incorporation (mean cpm ± SEM) by E-11*	Percent BLAST- 2 ⁺ B cells [‡]	AFC per culture [§]
Exp. 1				
B + media	—, 5		1	110
B + T + PWM				3,250
B + E-11		665 ± 231	2	150
B _{TNP} + media			1	20
B _{TNP} + T + PWM				1,625
B _{TNP} + E-11		6,752 ± 475	46	1,470
B + media	3, 6		1	10
B + T + PWM				2,300
B + E-11		267 ± 118	1	15
B _{TNP} + media			1	10
B _{TNP} + T + PWM				1,500
B _{TNP} + E-11		146 ± 73	44	25
Exp. 2				
B + media	3, 4		2	20
B + T + PWM				460
B + E-11		713 ± 174	3	35
B _{TNP} + media			4	40
B _{TNP} + T + PWM				500
B _{TNP} + E-11		1,645 ± 317	38	35
B + media	3, 5		2	30
B + T + PWM				4,000
B + E-11		695 ± 104	3	15
B _{TNP} + media			4	40
B _{TNP} + T + PWM				3,250
B _{TNP} + E-11		15,745 ± 3,192	34	2,250

* Proliferative responses of E-11 cells to each B cell population were assayed as described in Materials and Methods (8).

[‡] 10⁶ unmodified (B) or TNP-modified B cells (B_{TNP}) were cultured with final medium alone or with 0.25 × 10⁶ E-11 cells, and after 16 h, they were analyzed for BLAST-2 expression as described in Table I.

[§] AFC activity was determined using the reverse hemolytic plaque assay as described in Materials and Methods.

hypothesis, which we favor, is that the T cell receptor for antigen consists of at least two distinct binding sites, which appear to function sequentially. One is a high-affinity (or easily accessible) site, which recognizes foreign antigen alone. The other is a low-affinity (or normally inaccessible) MHC-binding site which is induced to increase its affinity for (or is exposed to bind) polymorphic class II determinants only after the binding site for foreign antigen has been engaged. This model is consistent with recent reports (10–11) that the T cell receptor may bind nominal antigen in the absence of MHC molecules, and is compatible with allosteric models (12, 13) of T cell receptor function that involve cooperativity between the nominal antigen and the MHC binding sites. Our experiments do not distinguish between a single α - β chain complex that contains both binding sites and the possibility that a second T cell surface receptor binds self MHC. Finally, the mechanism of Th cell receptor function proposed here makes teleologic sense. If the self MHC binding site of the Th cell receptor were of high affinity and readily accessible in the absence of foreign antigen, a continuous polyclonal in vivo activation of B cells (i.e., BLAST-2 induction) could result.

Summary

We used a cloned, TNP-specific, MHC-restricted, human Th cell line, E-11, and an assay of cognate Th-B cell interaction, BLAST-2 antigen expression on the B cell surface, to investigate the functional nature of the Th cell antigen receptor. We observed that E-11 induces BLAST-2 expression by resting B cells in a hapten-dependent, hapten-specific, but MHC nonrestricted manner. The implication of these results for the Th cell receptor are discussed.

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References

1. Kronenberg, M., G. Siu, L. E. Hood, and N. Shastri. 1986. The molecular genetics of the T cell antigen receptor and T cell antigen recognition. *Ann. Rev. Immunol.* 4:529.
2. Schwartz, R. H. 1986. Immune response genes of the murine major histocompatibility complex. *Adv. Immunol.* 38:31.
3. Crow, M. K., J. A. Jover, and S. M. Friedman. 1986. Direct T helper-B cell interactions induce an early B cell activation antigen. *J. Exp. Med.* 164:1760.
4. Kintner, C., and B. Sugden. 1981. Identification of antigenic determinants unique to the surfaces of cells transformed by Epstein-Barr virus. *Nature (Lond.)*. 294:459.
5. Thorley-Lawson, D. A., L. M. Nadler, A. K. Bhan, and R. T. Schooley. 1985. BLAST-2 (EBVCS), an early cell surface marker of human B cell activation, is superinduced by Epstein-Barr virus. *J. Immunol.* 134:3007.
6. Friedman, S. M., G. S. Thompson, and M. A. Principato. 1982. Allospecific human T cell lines and clones which mediate HLA-DR restricted helper activity. *J. Immunol.* 129:2451.
7. Goldberg, D., A. Green, A. B. Gottlieb, M. K. Crow, A. Lewison, and S. M. Friedman. 1985. Cloned allospecific human helper T cell lines induce an MHC-restricted proliferative response by resting B cells. *J. Immunol.* 135:1012.
8. Principato, M. A., G. S. Thompson, and S. M. Friedman. 1983. A cloned major histocompatibility complex restricted trinitrophenyl reactive human helper T cell line which activates B cell subsets via two distinct pathways. *J. Exp. Med.* 158:1444.
9. Guesdon, J.-L., T. Ternynck, and S. Avrameas. 1979. The use of avidin-biotin interaction in immunoenzymatic techniques. *J. Histochem. Cytochem.* 27:1131.
10. Carel, S., C. Bron, and G. Corradin. 1983. T cell hybridoma specific for a cytochrome C peptide: Specific antigen binding and interleukin-2 production. *Proc. Natl. Acad. Sci. USA.* 80:4832.
11. Rao, A., W. W.-P. Ko, S. J. Faas, and H. Cantor. 1984. Binding of antigen in the absence of histocompatibility proteins by arsonate-reactive T-cell clones. *Cell.* 36:879.
12. Blanden, R. V., D. B. E. C. Gill, R. Zidovetzki, and I. Pecht. 1980. Cooperativity in antigen binding as a potential element in Tc cell recognition. *Mol. Immunol.* 17:893.
13. Cleveland, W. L., and B. F. Erlanger, 1984. Hypothesis: The MHC-restricted Tc cell receptor as a structure with two multistate allosteric combining sites. *Mol. Immunol.* 21:1037.