T CELL PROLIFERATION INDUCED BY ANTI-SELF-I-A-SPECIFIC T CELL HYBRIDOMAS

Evidence of a T Cell Network

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We have been interested in the response of T cells to self MHC molecules (1, 2). Our laboratory and others have documented an in vitro interaction, which has been termed the autologous mixed lymphocyte reaction (AMLR) (3, 4) in humans, and the syngeneic mixed lymphocyte reaction (SMLR) in animals (5–8). This reaction has the attributes of a classical immune response: memory and specificity (9). Several groups (10–12) have found that T cells responding to self MHC antigens can be obtained from mice after immunization. Thus, these investigators found a high frequency of self-reactive T cells in lymph node cells from mice immunized with protein antigens.

The importance of self-reactive T cells in the generation and regulation of T cell diversity within the thymus had been suggested by Jerne (13). Regulatory control of this process is thought to occur in the thymus by selection against high-affinity self-reactive T cells. An additional level of control may operate in peripheral tissues, where self-reactive T cells are subjected to regulation by other cells of the immune system. While self-reactive T cells have been found in the periphery, the specificity of their proliferative response is determined intrathymically (14, 15), and the target antigens have been found to be self MHC class II molecules (16, 17). In the peripheral tissues, the cellular response to MHC antigens may be regulated by an idiotypic network, as is the humoral response to MHC antigens (18–20).

We present evidence that T cells from normal animals recognize some T cell hybridomas specific for self MHC class II antigens. This T cell response is not mediated by MHC antigens, and occurs only with T cells from mouse strains that express the MHC class II antigens recognized by the T cell hybridomas. This suggests that peripheral T cells may recognize selected idiotypic determinants expressed by a T cell receptor specific for their own MHC class II antigens.

Materials and Methods

Animals. AKR, BALB/c, and C57BL/6 mice, 6–8 wk-old were purchased from Charles River Laboratories, Inc. (Wilmington, MA). C3H/HeJ and the H-2 recombinant mice,

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B10.A(4R), B10.A(5R), B10.BR, and B10.MBR were obtained from The Jackson Laboratory (Bar Harbor, ME).

Monoclonal Antibodies. The hybridoma cell lines secreting the anti-I-A^b mAb 25-9-3S, the anti-I-A^d mAb MK-D6, the anti-I-A^k mAb 26-7-11S, the anti-Thy-1.1 mAb HO-22-1, the anti-Thy-1.2 mAb HO-13-4, and the anti-Lyt-2.2 mAb HO-2.2 were purchased from the American Type Culture Collection (Rockville, MD), and are currently maintained in our laboratory. The anti-Lyt-1.1 mAb (7-20.6/3), the anti-Lyt-1.2 mAb (CG16), and the anti-Lyt-2.1 mAb (49-31.1) were purchased from Accurate Chemical and Scientific Corp. (Westbury, NY). The anti I-A^b mAb 25-5-16 was a gift from the laboratory of Dr. David Sachs (National Institutes of Health, Bethesda, MD).

Mononuclear Cell Preparation. Mice were killed by cervical dislocation. Spleens were removed aseptically and teased apart in RPMI 1630 (Gibco Laboratories, Grand Island, NY) to prepare single-cell suspensions. Cells were collected by centrifugation and incubated for 5 min in ice with 5 ml of 0.83% ammonium chloride and 0.17 M Tris buffer, pH 7.2, to lyse erythrocytes. The cells were washed twice with RPMI 1630 and resuspended in culture medium for use.

Purified T cell populations were prepared according to the method described by Julius et al. (21), with minor modifications. Briefly, nylon wool columns were prepared and equilibrated with RPMI 1640 (Gibco Laboratories) supplemented with 5% heat-inactivated FCS, 20 mM Hepes, and 0.1% gentamycin (Gibco Laboratories), referred to as column medium. Nylon wool–nonadherent cells (NWNA)[1] were eluted with 15 ml warm column medium per 10^8 spleen cells (flow rate of one drop per second). 95% of these cells expressed the Thy-1.2 antigen. The cells were adjusted to 5×10^6 cells/ml in RPMI 1640 medium supplemented with 10% FCS, 20 mM Hepes, 2 mM L-glutamine, 5×10^{-5} M 2-ME (Eastman Organic Chemicals, Rochester, NY), 2.5% sodium pyruvate (Microbiological Associates, Walkersville, MD), and 0.1% gentamycin, referred to as complete culture medium (CCM).

Cell Culture. Allogeneic mixed lymphocyte cultures (MLC) were established in 25-cm^2 flasks (Falcon Labware, Oxnard, CA) by incubating 3.25×10^7 NWNA spleen cells from BALB/c mice with an equal number of γ-irradiated (3,000 rad, Cs source) allogeneic unfractionated spleen cells from C57BL/6 or C3H/HeJ in a total of 15 ml of CCM. Allogeneic MLC were incubated for 4 d in a 37°C 5% CO_2/95% humidified air atmosphere. Alloactivated T cell blasts were isolated after 4 d of MLR culture on a six-step Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient, as previously described (22). The T cell blasts were recovered at the 50–55% interface.

Generation of T Cell Hybridomas. Alloactivated T cell blasts isolated on Percoll gradients from allogeneic MLC were fused with the hypoxanthine/guanine phosphoribosyl transferase (HGPRT)-deficient AKR thymoma cell line, BW5147, at a 4:1 ratio using 40% PEG, mol wt 500–600 (Microbiological Associates). After 2–3 wk of culture in selective HAT medium, T cell hybridomas were transferred to 24-well culture plates (1 ml/well), expanded, and their reactivity was tested. Those T cell hybridomas of interest were cloned by plating cells at a concentration of 0.3 cells/well in the presence of 10^6 irradiated spleen cells from BALB/c mice.

T Cell Hybridoma Stimulation and IL-2 Assay. T cell hybridomas were stimulated according to the method described by Kappler et al. (23), with minor modifications. Briefly, 10^5 hybridoma cells were cultured in triplicate in 200 μl flat-bottom microtiter plates (2072; Falcon Labware), with or without 10^6 irradiated whole spleen cells in CCM, and incubated in a 5% CO_2/95% air environment of 37°C. After 24 h, 100 μl of supernatant was harvested and frozen at −20°C until assayed for IL-2 content according to the method described by Gillis (24) using the IL-2 dependent CTLL cell line. Briefly, assays were performed in triplicate using 100 μl culture supernatant and 100 μl of CCM containing 4×10^3 CTLL cells (provided by Dr. Steven Gillis, Immunex Corp., Seattle, WA). Cultures were incubated for 24 h. Proliferation of CTLL was measured during the

[1] *Abbreviations used in this paper:* CCM, complete culture medium; MLC, mixed lymphocyte culture; NWNA, nylon wool nonadherent cells.

TABLE I

IL-2 Production by Alloreactive T Cell Hybridomas Incubated with Irradiated Spleen Cells

Responder T cell hybridomas	IL-2 production by hybridomas cultured with irradiated spleen cells:		
	BALB/c	C57BL/6	C3H/HeJ
T1.117	0.2	2.7	0.3
T1.203	0.4	18.2	0.0
T1.301	0.8	7.9	0.3
T1.320	0.6	2.8	0.4
T1.321	0.5	7.0	0.0
T1.401	1.9	11.2	1.5
T1.426	2.8	15.3	—
T2.146	0.6	1.3	15.0
T2.205	3.7	3.0	10.4
T3.116	0.2	0.2	6.6
BW5147	0.3	0.2	0.1

T cell hybridomas were incubated with irradiated spleen cells for 24 h. IL-2 content is expressed as Δcpm $\times 10^{-3}$ [^{3}H]TdR incorporation by triplicate cultures of 4×10^3 CTLL cells. Background incorporation by CTLL plus media was 0.5×10^{-3} cpm. Significant values are underscored.

last 6 h of culture by adding 1 μCi of methyl-[^{3}H]thymidine, sp act 2 Ci/mM, (Amersham Corp., Arlington Heights, IL) in 1 μl to each microtiter well.

Stimulation of T Cells Using T Cell Hybridomas. T cell hybridomas were also tested for their capacity to stimulate splenic NWNA. Irradiated (3,500 rad) T cell hybridomas (10^4 in 0.1 ml CCM) were cocultured in round-bottom microtiter plates with 2.5×10^5 NWNA spleen cells in 0.1 ml CCM. The cultures were incubated for 3 d at 37°C in a humidified 5% CO_2/95% air environment. [^{3}H]Tdr incorporation was measured during the last 8 h of culture, as described above.

Immunofluorescent Analysis. Indirect immunofluorescent staining was performed as described by Reinherz et al. (25). Cells were analyzed on a modified cytofluorograph (FC 200; Ortho Diagnostics, Raritan, NJ).

Results

Establishment of Alloreactive T Cell Hybridomas. BALB/c splenic T cells were cultured with irradiated spleen cells from either C57BL/6 or C3H/HeJ mice. The T lymphoblasts generated in these cultures were isolated on Percoll gradients and fused to AKR thymoma cells BW5147. 40 hybrids were obtained from BALB/c cultures with C57BL/6 cells (T1 lines) and 42 hybrids from BALB/c cultures with C3H/HeJ cells (T2 and T3 lines). 8 of the 40 T1 hybrids and 3 of the 42 T2 and T3 hybrids produced IL-2 when cultured with irradiated spleen cells from C57BL/6 or C3H/HeJ mice, respectively. A representative experiment is shown in Table I. The T cell hybridomas T1.203, T1.321, T1.426 have been studied more than 10 times, and all T cell hybridomas have been studied at least three times, with similar results over the course of 1 yr. The parental thymoma line, BW5147, did not produce IL-2 constitutively or when cultured with either C57BL/6 or C3H/HeJ spleen cells. Similarly, the irradiated

TABLE II

IL-2 Production by H-2^b-reactive T Cell Hybridomas: Mapping of Stimulatory MHC Determinant

Stimulators	MHC								IL-2 production by T cell hybridomas		
	K	A	B	J	E	C	S	D	T1.203	T1.321	T1.426
C57BL/6	b	b	b	b	b	b	b	b	<u>14.9</u>	<u>5.9</u>	<u>2.8</u>
BALB/c	d	d	d	d	d	d	d	d	0.1	0.0	0.0
C3H/HeJ	k	k	k	k	k	k	k	k	0.1	0.0	0.0
B10.A(5R)	b	b	b	k	k	d	d	d	<u>14.1</u>	<u>7.4</u>	<u>2.7</u>
B10.A(4R)	k	k	b	b	b	b	b	b	0.0	0.2	0.0
B10.MBR	b	k	k	k	k	k	k	q	0.1	0.0	0.0
Media									0.0	0.0	0.0

T cell hybridomas were incubated with irradiated spleen cells for 24 h. IL-2 content is expressed as Δcpm $\times 10^{-3}$ [^{3}H]TdR incorporation by triplicate cultures of 4×10^3 CTLL cells. Background incorporation by CTLL plus media was 0.6×10^{-3} cpm. Significant values are underscored; note correlation with H-2A^b haplotype.

spleen cells did not produce IL-2 when cultured alone or with the AKR thymoma cells.

Mapping MHC Antigens that Stimulated Production of IL-2 by T Cell Hybridomas. Six T cell hybridomas, three responsive to C57BL/6 (T1.203, T1.321, and T1.426) and three responsive to C3H/HeJ (T2.146, T2.205, and T3.116) were selected for further study after cloning by limiting dilution. Spleen cells from a series of mice recombinant at the H-2 locus were used to identify the antigen recognized by these alloreactive T cell hybridomas. T cell hybridomas T1.203, T1.321, and T1.426, activated by H-2^b spleen cells, recognized determinants on I-A^b gene products (Table II). Similarly, the T cell hybridomas T2.146, T2.205, and T3.116, which respond to H-2^k spleen cells, are reacting to I-A^k determinants, as confirmed by their pattern of reactivity with H-2 recombinant mice (Table III).

Inhibition of IL-2 production by mAbs against I-A antigens also supports the involvement of these molecules in the activation of the hybrids. Thus, mAb 25-5-16, directed against I-A^b antigens, inhibited IL-2 production by the T cell hybridomas T1.203 and T1.321, reactive with H-2^b spleen cells, but had no effect on the production of IL-2 by the T cell hybridoma, T2.205, which was activated by H-2^k spleen cells (Fig. 1). Similarly, mAb specific for I-A^k antigens inhibited IL-2 production by the T cell hybridomas, T2.205, T2.146, and T3.116 by >80%, but not IL-2 production by T1.203 (data not shown).

Surface Antigens Expressed by Alloreactive T Cell Hybridomas. Surface antigens expressed by the T cell hybridomas, T1.203 and T2.205, as well as AKR thymoma cells, were characterized using mAb specific for a number of lymphocyte surface antigens. Both T cell hybridomas expressed the Thy-1.2 antigen that was not expressed by the AKR thymoma line. Neither T cell hybridomas nor the AKR thymoma line expressed detectable I-A surface antigens (Fig. 2). The AKR thymoma line and the T cell hybridomas expressed the Thy 1.1 and the H-2^k antigen (data not shown). Neither hybrids nor AKR thymoma expressed Lyt-1.2 or Lyt-2.2 antigens (data not shown).

TABLE III

IL-2 Production by H-2^k-reactive T Cell Hybridomas: Mapping of Stimulatory MHC Determinant

Stimulators	MHC								IL-2 production by T cell hybridomas		
	K	A	B	J	E	C	S	D	T2.146	T2.205	T3.116
C3H/HeJ	k	k	k	k	k	k	k	k	15.0	3.2	3.3
C57BL/6	b	b	b	b	b	b	b	b	0.5	0.7	0.8
BALB/c	d	d	d	d	d	d	d	d	0.0	0.0	0.8
B10.BR	k	k	k	k	k	k	k	k	10.6	4.3	7.1
B10.MBR	b	k	k	k	k	k	k	q	9.7	4.9	—
B10.A(4R)	k	k	b	b	b	b	b	b	10.0	3.8	4.1
B10.A(5R)	b	b	b	k	k	d	d	d	0.2	0.6	0.5
Media									0.2	0.3	0.6

T cell hybridomas were incubated with irradiated spleen cells for 24 h. IL-2 content is expressed as Δcpm $\times 10^{-3}$ [^{3}H]TdR incorporation by triplicate cultures of 4×10^3 CTLL cells. Background incorporation by CTLL plus media was 0.6×10^{-3} cpm. Significant values are underscored; note correlation with H-2A^k haplotype.

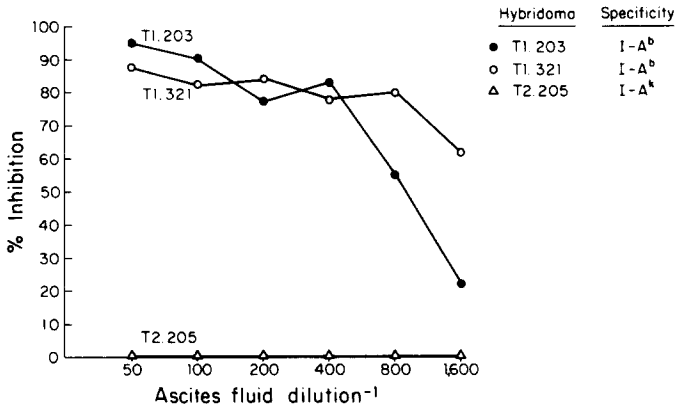

FIGURE 1. Inhibition of IL-2 production by mAb 25-5-16 specific for I-A^b. Various amounts of 25-5-16 containing ascitic fluid were added to the indicated final concentrations in the assay for IL-2 production.

Certain Alloreactive T Cell Hybridomas Stimulate Proliferation of T Cells from Target Mouse Strains. The capacity of alloreactive T cell hybridomas to stimulate spleen cells from donor, target, or third party mouse strains was examined. Two of the T cell hybridomas (T1.203 and T1.321) activated by C57BL/6 spleen cells stimulated thymidine incorporation by splenic T cells from C57BL/6 mice, but not by T cells from third party (C3H/HeJ) or parental, (BALB/c) mice. The AKR thymoma cells showed no specific stimulation of T cells from any mouse strain. The T cell hybridomas T1.203 and T1.321 have been studied more than seven times and have consistently stimulated splenic T cells from C57BL/6 mice. The stimulation index ([stimulated cpm]/[background cpm]) in these experiments varied from 5 to 30. A representative experiment is shown in Table IV.

Similarly, two of three T cell hybridomas activated by C3H/HeJ spleen cells stimulated splenic T cells from C3H/HeJ mice but not splenic T cells from third party (C57BL/6) or parental (BALB/c) mice. The T cell hybridomas T2.146

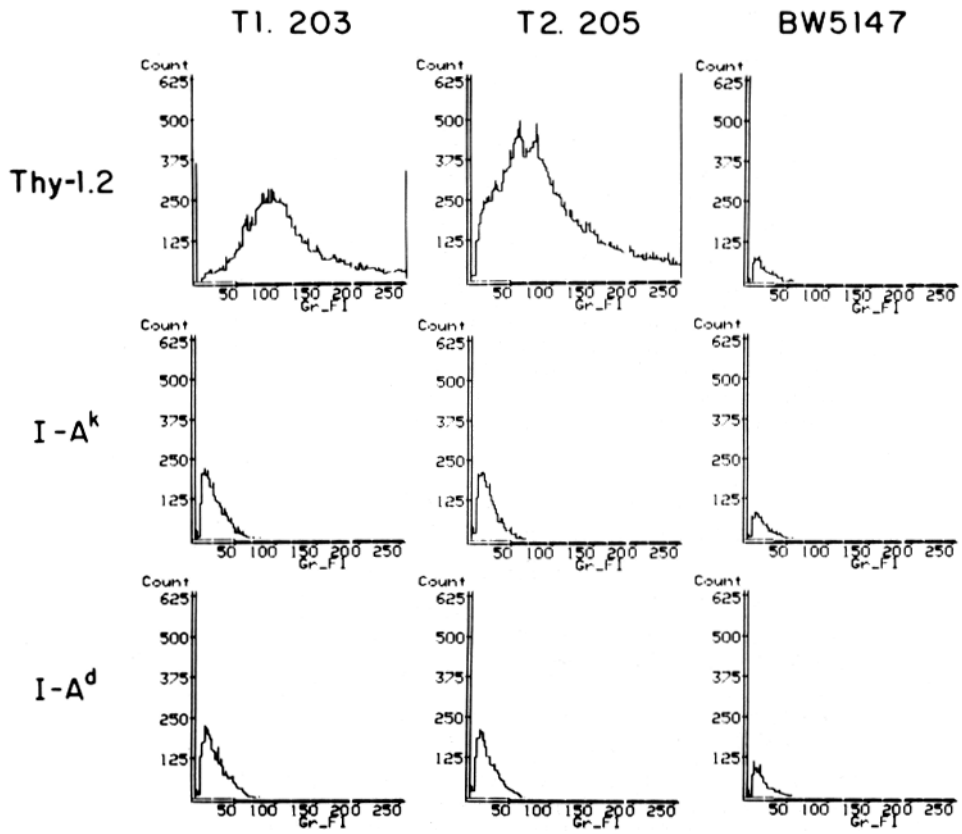

FIGURE 2. Surface expression of Thy-1.2, I-A^k and I-A^d antigens. T cells hybridomas T1.203, T2.205, and the parental thymoma BW5147 were incubated with mAbs specific for these antigens and analyzed with an Ortho cytofluorgraph. Results are expressed as cell number vs. log fluorescence intensity.

TABLE IV

C57BL/6 T Cell Proliferation Is Stimulated by Certain I-A^b-reactive T Cell Hybridomas

Source of responder T cells	[^{3}H]TdR incorporation in presence of irradiated T cell hybridomas				
	T1.203	T1.321	T1.401	T1.426	BW5147
C57BL/6	<u>20.3</u>	<u>13.2</u>	2.5	3.0	7.6
C3H/HeJ	1.3	3.0	3.0	3.2	4.0
BALB/c	2.3	6.0	4.4	2.3	7.0

2.5×10^5 responder T cells were incubated with 10^4 irradiated (3,500 rad) T cell hybridomas for 72 h. Results are expressed as Δcpm $\times 10^{-3}$ [^{3}H]TdR incorporation by triplicate cultures during the last 8 h of culture. Background incorporation (cpm) of [^{3}H]TdR by T cells: C57BL/6, 1.2; C3H/HeJ, 1.6; BALB/c, 1.3. Significant values are underscored.

TABLE V

C3H/HeJ T Cell Proliferation Is Stimulated by Certain I-A^k-reactive T Cell Hybridomas

Source of responder T cells	[^{3}H]TdR incorporation in presence of irradiated T cell hybridomas			
	T2.146	T2.205	T3.116	BW5147
C57BL/6	0.3	4.7	0.6	2.5
C3H/HeJ	7.2	12.1	0.0	2.7
BALB/c	1.4	2.5	0.2	2.8

2.5×10^5 responder T cells were incubated with 10^4 irradiated (3,500 rad) T cell hybridomas for 72 h. Results are expressed as Δcpm $\times 10^{-3}$ [^{3}H]TdR incorporation by triplicate cultures during the last 8 h of culture. Background incorporation (cpm) of [^{3}H]TdR by T cells: C57BL/6, 1.0; C3H/HeJ, 1.1; BALB/c, 1.3. Significant values are underscored.

TABLE VI

T Cell Proliferation Induced By T Cell Hybridomas: Effect of Anti-MHC mAbs

Source of responder T cells	Stimulator T cell hybridomas	[^{3}H]TdR incorporation in presence of mAb specific for:				
		I-A^k	I-A^b	K^k	K^b	Media
C57BL/6	T1.203	16.1	13.5	15.9	14.3	15.7
C3H/HeJ	T2.205	8.1	6.7	4.5	6.7	5.8

2.5×10^5 responder T cells were incubated with 10^4 irradiated (3,500 rad) T cell hybridomas for 72 h. Results are expressed as Δcpm $\times 10^{-3}$ [^{3}H]TdR incorporation by triplicate cultures during the last 8 h of culture. MAb were culture supernatants used at a final concentration of 1:20. Background incorporation (cpm) of [^{3}H]TdR by T cells: C57BL/6, 0.9; C3H/HeJ, 0.7.

and T2.205 were studied at least three times and have stimulated splenic T cells from C3H/HeJ mice from 4- to 20-fold. A representative experiment is shown in Table V. Immunofluorescent analysis of the responding population showed that the responding cell is a T cell, because >95% of day 3 blasts stained with an anti-Thy-1.2 mAb. Thus, certain alloreactive T cell hybridomas showed reciprocal interactions with the stimulating mouse strain; alloreactive T cell hybridomas responded to and stimulated lymphocytes from the same mouse strain.

The response of T cells from target mouse strains, to which the irradiated T cell hybridomas are specific, was not inhibited by several mAb against MHC class I or class II antigens (Table VI). Several lines of evidence suggest that the stimulatory determinants on these T cell hybridomas are related to their fine specificity. First of all, only certain alloreactive T cell hybridomas expressed this activity. Furthermore, the stimulatory T cell hybridomas did not express MHC class II surface antigens, and mAb against MHC class II antigens did not inhibit this reaction. Thus, the activation of T cells by certain alloreactive T cell hybridomas may reflect their recognition of idiotypic determinants on the anti-self-I-A receptor, suggesting an idiotype-antiidiotype interaction.

Discussion

We have obtained a number of alloreactive murine T cell hybridomas by fusing BALB/c lymphoblasts generated in MLR culture with irradiated spleen cells from C57BL/6 or C3H/HeJ mice with the AKR thymoma line BW5147. The T cell hybridomas produced IL-2 when cultured with irradiated spleen cells from the mouse strain used as source of stimulatory cells in MLR, but not with other irradiated allogeneic spleen cells, or with cells from the parental BALB/c strain. Using a series of MHC-recombinant mice we have shown that the C57BL/6-reactive hybrids recognize the I-A^b antigen, while the C3H/HeJ-reactive hybrids respond to I-A^k antigens. Inhibition studies with anti-I-A^b and -I-A^k mAbs supported these findings. The reactivity of these hybrids with I-A antigens is not surprising, since the screening method chosen, IL-2 production, may have selected for MHC class II–reactive hybrids. Additionally, the predominant stimulating antigen in the MLR has been reported to be I region–encoded (26–29).

The key finding in this study is that some anti-I-A^b (C57BL/6) and anti-I-A^k (C3H/HeJ) -reactive T cell hybridomas stimulated the proliferation of T cells from C57BL/6 and C3H/HeJ mouse strains, respectively. However, these T cell hybridomas did not stimulate proliferation of T cells from parental BALB/c or third party mouse strains. Thus, there is a reciprocal interaction between these alloreactive T cell hybridomas and splenic T cells from the mouse strain expressing the I-A antigen that activates the hybrid. This suggests the existence, in naive mice, of T cells that recognize anti–self–MHC class II–reactive lymphocytes.

To understand the mechanism of this interaction, it is crucial to know the antigens on the alloreactive T cell hybridomas that activate allogeneic T cells. The stimulation of splenic T cells by these anti-I-A hybrids does not appear to be due to the histocompatibility differences between the hybrids and the proliferating T cells. Cytofluorometric analysis of the hybrids showed no detectable surface MHC class II expression, while both the hybrids and the parental thymoma BW5147 expressed the K^k antigen. Furthermore, the stimulation of splenic T cells by these T cell hybridomas is not affected by anti–MHC class II mAbs. These findings are not compatible with a T cell response driven by MHC class I or II antigens.

All of the hybridomas studied secrete IL-2 in response to irradiated spleen cells, although only some stimulate appropriate T cells to proliferate. Thus, neither T1.401 nor T1.426 stimulates proliferation of C57BL/6 T cells, although both produce significant amounts of IL-2 in response to C57BL/6 spleen cells. Furthermore, these irradiated T cell hybridomas cultured with nylon wool–purified T cells do not secrete detectable amounts of IL-2, as measured using the IL-2 assay. This differential stimulatory capability may reflect the hybridomas' reactivity with different epitopes on I-A molecules. Indeed, there is evidence (30) that T cell hybridomas can recognize multiple determinants on I-A molecules.

For these reasons, we believe it is likely that the T cell proliferative response is directed to idiotypic determinants on the recognition structure of the MHC class II–reactive hybrids. Of particular interest is the finding that these T cells exist in unmanipulated animals, suggesting the possibility that they have immunoregulatory functions. Although we do not yet have molecular evidence that

the stimulatory determinant is an idiotype expressed by the T cell receptor on these hybridomas, this possibility best explains our observations.

Our hypothesis that T cells may interact with idiotypic determinants expressed by other T cells is supported by previous studies. It has been shown (31, 32) that (A × B) F_1 rats become resistant to lethal graft-vs-host (GVH) reaction induced by A lymphocytes if they are first inoculated with low numbers of A lymphocytes. However, these rats remain susceptible to GVH induced by the other parent (B) lymphocytes. This GVH resistance is transferable and thought to be mediated by T cells specific for idiotypic determinants expressed on the anti-MHC receptors of the immunizing parental T cell population. Other investigators (33, 34) have found that T cells can distinguish in vitro autologous T cell blasts directed to different allo–MHC class II antigens in a secondary response. Both these studies suggest that T cells with receptors for MHC class II antigens may express idiotypic determinants that can be recognized by other T cells.

A decade ago, Jerne suggested (13) that the T cell repertoire is created by diversification of progenitor T cells with self-reactivity. This hypothesis implies that the recognition of anti–self–MHC class II reactivity is necessary within the immune system. If self-reactivity is the foundation upon which T cell diversity is built, self-reactivity must be precisely regulated. This may occur through idiotype-antiidiotype interactions, analogous to the idiotypic network among B cells and their products. Recent studies (35) suggest that autoreactive T cell clones are involved in a T cell network. Our data suggest that T cells specific for idiotypic determinants on anti–self-I-A–reactive lymphocytes exist in vivo. Such cells may participate in the control of autoreactivity by regulating lymphokine production during anti–self MHC reactions, and may ultimately determine the level of help. The availability of cloned lines of cells will allow a detailed analysis of these cell interactions.

Summary

Allo-I-A–reactive T cell hybridomas were generated from MLR-activated lymphoblasts. Cloned hybridomas T1.203, T1.321, and T1.426 were stimulated by $I\text{-}A^b$ determinants, as shown by their ability to secrete IL-2 in response to a panel of MHC-recombinant mice. T2.146, T2.205, and T3.116 were found to be specific for $I\text{-}A^k$ determinants using a similar panel of MHC-recombinant mice. Inhibition of IL-2 secretion by anti-I-A mAb confirmed these data. Some $I\text{-}A^b$-specific hybrids stimulated the proliferation of T cells from C57BL/6 ($H\text{-}2^b$) mice. Similarly, some $I\text{-}A^k$-specific hybrids stimulated the proliferation of T cells from C3H/HeJ ($H\text{-}2^k$) mice. These hybrids expressed no detectable surface I-A, and the stimulation of T cells was not inhibited by anti-I-A mAb. These results are consistent with the hypothesis that normal mice possess a population of T cells responsive to idiotypic determinants on anti–MHC class II T cell receptors.

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