Brief Definitive Report

ANTIGEN-REACTIVE T CELLS CAN BE ACTIVATED BY AUTOLOGOUS MACROPHAGES IN THE ABSENCE OF ADDED ANTIGEN*

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The syngeneic or autologous mixed lymphocyte reaction (AMLR) is a form of self recognition represented by the proliferation of T cells when they are appropriately cultured with autologous, non-T cells (1, 2). Although workers in several laboratories have documented this phenomenon, the relationship of cells participating in the AMLR and those required for relevant immune function, and the significance of this reaction are controversial. These two points are linked, because any conclusion concerning the relevance of the in vitro AMLR to in vivo immunologic communication depends on a delineation of the function of autologous responder and stimulator cells. We have reported that the AMLR actually represents proliferation among two distinct population of T cells in response to signals from distinct stimulator cells (3, 4). One population of T cells is activated by stimulators present in autologous B cell-enriched, macrophage (Mϕ)-depleted cells, whereas another is induced to proliferate by signals from a subpopulation of autologous Mϕ. Furthermore, data were presented demonstrating that those Mϕ stimulating in the AMLR contained antigen-presenting cells, whereas T cells proliferating in response to these Mϕ were required for proliferative reactivity and lymphokine synthesis induced by soluble antigens. Here we present data that directly demonstrate that T cells responsive to autologous Mϕ in the absence of added antigen are themselves antigen reactive.

Materials and Methods

Peripheral blood mononuclear cells (PBMC) were obtained from normal volunteers and four patients with common variable immunodeficiency. The patients were selected on the basis of their failure to demonstrate a positive delayed hypersensitivity skin test (i.e., >5 × 5 mm induration) to any of five antigens (mumps, intermediate-strength purified protein derivative (PPD), *Candida albicans*, trichophyton, and streptokinase-streptodornase). These patients also had total serum immunoglobulin levels of <3.5 mg/ml.

Populations of PBMC enriched for T cells (92 ± 4% T, 3 ± 1% Ig-bearing, 4 ± 2% esterase-positive), B cells (>90% Ig-bearing), and Mϕ (>95% esterase-positive) were obtained as previously described (3). Proliferative reactivity to autologous stimulator cells and soluble antigens was measured over a 6–9-d culture in 10% heat-inactivated autologous serum using

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two ratios of responder to stimulator cells (2:1, 1:1) or three concentrations of soluble antigen (3). The results are reported as maximal reactivities.

Negative selection of T cells proliferating in response to autologous stimulator cells was performed using 5-bromo-2-deoxyuridine (BrdUrd; Sigma Chemical Co., St. Louis, Mo.) as described (3).

To positively select for T cells responsive to autologous Mϕ, T cells were coated with the monoclonal antibody, T-29, and then allowed to form rosettes with IgG goat anti-mouse IgG (N. L. Cappel Laboratories, Inc., Cochranville, Pa.) covalently linked to bovine erythrocytes. T-29 is a κ,2b immunoglobulin which, by immunofluorescence using phase-contrast microscopy and cytotoxicity, detects a determinant present on 9.8 ± 2.1% of the peripheral blood T cells from several HLA-DR-disparate individuals. The specificity of this antibody has been previously described (3). The positive-selection procedure used 10 million T cells incubated with 100 μg of affinity column-purified T-29 for 30 min at 4°C. The cells were then washed, mixed with an equal volume of the goat anti-mouse IgG-conjugated bovine cells for 5 min at 4°C, centrifuged at 500 rpm, and incubated for 30 min at 4°C. Rosette-forming cells were then pelleted through Ficoll/Hypaque (specific gravity = 1.092) at room temperature and the erythrocytes were lysed with Tris-buffered ammonium chloride. When assayed by indirect immunofluorescence, the pellet contained 86.6 ± 3.9% T-29$^+$ cells. Depletion of T-29$^+$ cells in the population remaining at the top of the Ficoll-Hypaque was variable and never complete.

Results

Previously we reported that cytolytic treatment of T cells with the monoclonal antibody T-29 diminished reactivity to soluble antigen and autologous Mϕ (3). This finding could indicate either that both these reactivities were mediated by the same population of T-29$^+$ cells, or that they represented properties of distinct T cell populations, both of which displayed the T-29 determinant. The negative-selection experiments outlined in Table I directly support the first possibility. BrdUrd and light treatment of T cells proliferating in response to autologous Mϕ markedly diminished their subsequent proliferative response to the soluble antigens *C. albicans* and PPD. The subsequent reactivity of these cells to autologous B-enriched stimulators was not decreased (3). The noted diminution in antigen reactivity among populations depleted of Mϕ-responsive T cells did not represent a nonspecific effect of BrdUrd and light, because negative selection of T cells proliferating in response to autologous B cells did

TABLE I

Reactivity of Negatively Selected T Cells to Autologous Mϕ and Antigens

Responding populations		Maximal reactivity			
		Media	Mϕ	*C. albicans*	PPD
				cpm	
A. Negative selection of Mϕ-responsive T					
Experiment 1	T + Mϕ + BrdUrd, no light	909	7,719	6,373	12,611
	T + Mϕ + BrdUrd, light	868	1,008	1,078	964
Experiment 2	T + Mϕ + BrdUrd, no light	1,324	8,739	14,496	34,279
	T + Mϕ + BrdUrd, light	4,012	3,146	4,763	6,285
B. Negative selection of "B"-responsive T					
Experiment 1	T + B + BrdUrd, no light	3,462	24,580	12,736	24,097
	T + B + BrdUrd, light	3,901	37,449	16,867	22,301
Experiment 2	T + B + BrdUrd, no light	1,642	3,612	7,370	ND
	T + B + BrdUrd, light	2,819	3,100	13,670	ND

T cells were cultured with either autologous Mϕ (A) or autologous B-enriched populations (B) and BrdUrd was added as previously described (4). One-half of the cultures were exposed to cool, white light. All cultures were washed and compared at comparable concentrations of viable cells for their reactivity to media only, two concentrations of autologous Mϕ, or three concentrations of *C. albicans* and PPD. Reactivity was measured by the incorporation of [^{3}H]TdR into DNA and is expressed as maximal counts per minute. ND, not done.

not diminish the subsequent reactivity to either *C. albicans* or PPD. The subsequent response of these negatively selected cells to autologous B cells was diminished by a mean of 51% (data not shown).

These negative-selection experiments, as well as those previously reported using cytolysis with T-29, do not allow a decision as to whether the Mϕ-responsive, $T\text{-}29^+$ population of T cells is simply required for antigen reactivity or whether the T cells themselves are antigen reactive. To decide between these two alternatives, we used a positive-selection technique to obtain populations enriched for $T\text{-}29^+$ T cells. T-enriched populations were coated with either T-29 or a control monoclonal antibody, T-15, which reacts with 15% of T cells not detected by T-29. The coated cells were then allowed to form rosettes with goat anti-mouse IgG conjugated to ox erythrocytes. Rosette-forming cells were separated from cells not forming rosettes by centrifugation through Ficoll-Hypaque (Table II) To determine reactivity to soluble antigens, the pellet and interface were reconstituted with comparable concentrations of autologous Mϕ (3%) to ensure that any difference in reactivity was not limited by antigen-presenting cells. When compared with T cells remaining on top of the Ficoll-Hypaque (interface, experiments 1 and 2, Table II, A), populations enriched for $T\text{-}29^+$ cells (pellet, 86.6 ± 3.9% $T\text{-}29^+$) were also enriched in their reactivity to autologous Mϕ and to the two soluble antigens tested. This enrichment did not represent nonspecific sedimentation of reactive cells through the Ficoll-Hypaque. Fractionation of T cells coated with T-15 did not result in any enrichment of proliferative reactivity to either autologous Mϕ or soluble antigen among the pelleted cells (Table II, B).

All the experiments designed to investigate the relationship among T-29 positivity, antigen reactivity, and reactivity to autologous Mϕ required several in vitro manipulations. If the correlations we have shown in vitro are relevant to interactions between T and non-T cells required for antigen reactivity in vivo, a quantitative deficiency of $T\text{-}29^+$, Mϕ-responsive cells may exist, *de novo*, in some patients with cutaneous anergy. The data presented in Table III outline the results obtained in four

TABLE II

Reactivity of Positively Selected Cells to Autologous Mϕ and Antigens

Responding populations	Maximal reactivity			
	Media	Mϕ	*C. albicans*	PPD
		cpm		
A. Selected with T-29				
Experiment 1				
Pellet	3,842	15,189	20,388	21,511
Interface	3,225	3,227	4,994	9,878
Experiment 2				
Pellet	8,599	50,009	45,709	51,238
Interface	5,596	6,711	19,976	13,863
B. Selected with T-15				
Experiment 1				
Pellet	335	444	746	1,397
Interface	4,296	ND	34,597	27,873
Experiment 2				
Pellet	3,977	5,533	11,191	16,880
Interface	5,039	28,342	40,168	109,019

T cells were coated with the T cell-specific, monoclonal antibody T-29 (A) or T-15 (B). The coated cells were then allowed to form rosettes with goat antimouse IgG covalently linked to ox erythrocytes. Rosette-forming cells (pellet) were then separated from cells not forming rosettes (interface) by centrifugation through Ficoll-Hypaque. Each population was then compared for its proliferative reactivity to media only, two concentrations of Mϕ, or three concentrations of *C. albicans* or PPD. Results are expressed as outlined in Table I. ND, not done.

patients with common variable immunodeficiency who failed to respond to any of five antigens used to elicit delayed hypersensitivity. A marked (90%) reduction in the frequency of T-29$^+$ cells in these patients was accompanied by a marked decrease in the proliferative response to both autologous Mϕ and *C. albicans*. Reactivity to autologous B cells was relatively spared (50% reduction), as was reactivity to allogeneic stimulator cells (data not shown). It should be noted that the subpopulation of Mϕ that acts as a stimulator in the AMLR was quantitatively normal in these patients, suggesting that the defect in the Mϕ-responsive portion of AMLR resided in the responder and not in the stimulator population (data not shown).

Discussion

Specific activation of proliferating and helper T cells requires that they not only recognize nominal antigenic determinants but also immune response (Ir)-associated (Ia) glycoproteins displayed by antigen-presenting cells (5–7). The results of experiments performed in murine chimeras indicate that the phenotypic expression of T cell-recognition units for specific Ia determinants is acquired during their exposure to antigen-presenting cells in the thymus (8–10). Within the thymic environment of a normal host, antigen-reactive T cells are "taught" to recognize self Ia displayed by autologous, antigen-presenting cells. The AMLR represents a form of self recognition occurring in peripheral lymphoid tissue. Our studies indicate that a portion of the cells involved in this self recognition are functionally related to those involved in the self recognition initiated in the thymus. We have previously shown that antigen-presenting cells can serve as one of the stimulators in the AMLR. The data presented here indicate that T cells responsive to these Mϕ are themselves antigen reactive. In other words, the AMLR occurring between peripheral Mϕ and T cells is similar to self recognition initiated in the thymus in that it represents interactions between antigen-presenting and antigen-reactive cells.

Although the responder and stimulator cells involved in a portion of the AMLR are functionally similar to those involved in the acquisition of Ir gene restriction in the thymus, it is not known whether the proliferation of peripheral T cells exemplified

TABLE III

Reactivity of T Cells from Anergic Patients to Autologous Stimulator Cells and Conventional Antigen

	Positive cells		Reactivity to		
	Total T	T-29$^+$ T	B	Mϕ	*C. albicans*
	%				
A. Normals (15)	75	10	7.1	4.7	9,599
Mean ± SE	±4	±2	±1.2	±1.1	±1,120
B. Patients					
1	48	0	5.0	1.1	260
2	56	1	3.1	0.3	21
3	54	1	4.3	0.8	437
4	49	1	2.1	0.9	887
Mean ± SE	50.5	0.8	3.6	0.8	401
	±1.3	±0.3	±0.6	±0.2	±182

PBMC from 15 normal individuals (A) and 4 anergic patients (B) were compared for (*a*) the frequency of E rosette forming (i.e., total T), (*b*) the frequency of T cells detected by immunoflorescence with the T cell-specific, monoclonal antibody T-29 (T-29$^+$ T), (*c*) their proliferative reactivity to two concentrations of either autologous B-enriched cells or Mϕ, and (*d*) their proliferative response to three concentrations of *C. albicans*. Reactivity to autologous B or Mϕ is presented as maximal stimulation index. Reactivity to *C. albicans* is presented as Δ counts per minute (i.e., maximal cpm in cultures with antigen; cpm in cultures with media only).

by the AMLR represents a process involved in maintaining this restriction. Experiments using murine chimeras suggest that, although Ir restriction is initiated in the thymus, it may be perpetuated by subsequent interactions between antigen-reactive and antigen-presenting cells in the periphery (11, 12). The phenotypic expression of Ir gene restriction involves thymocyte recognition of Ia determinants displayed by thymic antigen-presenting cells. It has not been definitively established that the AMLR occurring between Mϕ and T cells requires comparable recognition of Ia.

If the AMLR between antigen-presenting and antigen-responsive cells represents physiologic self recognition required to maintain the immunologic integrity of peripheral T cells, one might then question the immunologic function and specificity of the remaining Mϕ-unresponsive T cells. We have not been able to demonstrate that these cells proliferate or synthesize lymphokines in response to the three soluble antigens tested (*C. albicans*, PPD, and collagen). However, they can (*a*) regulate immunoglobulin production, (*b*) proliferate in response to alloantigens, and (*c*) function as cytotoxic effector cells for allogeneic targets (P. Hausman and J. Stobo, unpublished observations). At least a portion of helper cells that regulate immunoglobulin production may recognize immunoglobulin idiotypes displayed by B cells, and activation of suppressor T cells may not require cellular presentation of antigen by Mϕ (13–15). Similarly, T cell reactivity to alloantigens may not require Ir-restricted presentation of antigen by Mϕ, but require accessory cells only as a source of soluble amplifying materials (Interleukin 1) (16, 17). Therefore, the Mϕ-unresponsive T cells do exhibit immune reactivity. However, their function may not be restricted by the same genetic constraints that govern the interaction between antigen-reactive T cells and antigen-presenting Mϕ, an interaction that may be represented by the AMLR to Mϕ.

It has been postulated that interactions between reactive T cells and either soluble antigen alone or antigen-presenting cells alone is not sufficient to induce their proliferation (18). This is supported by the demonstration that soluble antigen by itself cannot induce substantial proliferation among reactive T cells in the absence of Mϕ (5–7). Our studies indicated that autologous antigen-presenting cells can, in the absence of added antigen, induce proliferation among antigen-reactive T cells. These findings not only indicate that at least a portion of the AMLR represents interactions among immunologically relevant cells but also raise interesting questions concerning the nature of activating determinants and recognition units that govern interactions between antigen-reactive T cells and antigen-presenting Mϕ.

Summary

T cells responsive to macrophages (Mϕ) in the autologous mixed lymphocyte reaction (AMLR) contain those cells that can be induced to proliferate by soluble antigens. Negative selection (5-bromo-2-deoxyuridine and light) of T cells activated by autologous Mϕ also removed those cells required for reactivity to *Candida albicans* and purified protein derivative. Positive selection of T cells responsive to autologous Mϕ yields a population that is simultaneously enriched in antigen reactivity. Some patients demonstrating cutaneous anergy and diminished in vitro blast transformation in response to soluble antigen also lack T cells responsive in the AMLR to Mϕ. When considered in conjunction with previously reported data, these findings indicate the AMLR occurring between T cells and Mϕ in the absence of soluble antigen represents self recognition occurring between antigen-reactive T cells and antigen-presenting Mϕ.

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