

IDIOTYPE-LIKE DETERMINANTS ON HUMAN T
LYMPHOCYTES ALLOACTIVATED IN
MIXED LYMPHOCYTE CULTURE*

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We have previously (1-3) shown that human T lymphocytes that have been primed to allogeneic stimulators in mixed lymphocyte culture (MLC), acquire the capacity not only of expressing HLA-D and DR antigens but also of stimulating autologous MLC responses (AMLR). This observation has led us to propose that the Ia-like antigens expressed by primed T cells may be associated with idiotypes that trigger the anti-idiotypic lymphocyte response, and that by analogy with the antibody system, such anti-idiotypes may regulate the T cell immune response (3).

Because previous studies, however, have shown that resting T lymphocytes respond in AMLR to cells expressing HLA-D/DR antigens, such as B cells and monocytes (4-6), proof to the effect that the autologous MLC reactivity to alloactivated T cells is truly idiotypic (i.e., receptor-) specific, rather than specific for self-Ia, is required. The present experiments demonstrate that T cells primed against autologous allostimulated T lymphoblasts (AAL) display specific "memory" responses against the original autostimulator in the primed lymphocyte test (PLT) and fail to exhibit secondary reactivity to autologous T lymphoblasts (AL) primed against a different HLA-D/DR specificity.

Materials and Methods

Lymphocyte Preparations. Blood was obtained from healthy volunteers with a mean age of 32 yr. Peripheral blood lymphocytes (PBL) containing <5% granulocytes were isolated by Ficoll-Hypaque gradient centrifugation. The suspensions were depleted of monocytes by removal of cells adhering to petri dishes. T cells were separated by the thrombin-nylon wool method (7). Further purification of T cells was accomplished by incubating 1×10^7 lymphocytes with $1 \mu\text{g}$ of mouse monoclonal OKT-3 antibody (Ortho Pharmaceutical, Raritan, NJ), and subsequently rosetting the T lymphocytes with sheep erythrocytes (SRBC) coated with purified anti-mouse IgG (8). After lysing the SRBC, T cells were washed and resuspended in RPMI 1640 culture medium supplemented with glutamine, antibiotics, and pooled normal male serum. Immunofluorescence determination using a Spectrum III Cytofluorometer (Ortho Diagnostics) showed that >95% of the cells contained by these suspensions were OKT-3 positive.

Priming of OKT-3-positive T Cells against AAL. OKT-3⁺ T cells obtained from fresh PBL were used as responders (1×10^7 cells in 10 ml of medium) and were primed in 9-d MLC with an equal number of irradiated AAL, representing the stimulating cells. AAL were obtained from a 5-d MLC between the same responder and irradiated monocyte-depleted lymphocytes

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from a HLA-DR-half-identical allostimulator. Lymphoblasts were obtained from the 5-d culture by differential centrifugation on a 55% isotonic Percoll suspension (1.072 g/ml), followed by centrifugation through a 30% Percoll suspension for removal of dead cell debris (9). Cytofluorometric examination of AAL showed that virtually all cells were OKT-3⁺, Ia⁺ lymphoblasts that displayed no surface human Ig. The HLA-DR phenotype of AAL was indistinguishable from that expressed by fresh B lymphocytes from the same responder. For restimulation experiments, anti-AAL PLT were plated at a concentration of 5×10^4 cells/culture and challenged in triplicate reactions with an equal number of stimulating cells. Cultures were labeled with tritiated thymidine after 48 h of incubation and processed for liquid scintillation counting 18 h later (1-3).

Results

The aim of these experiments was to determine whether T lymphocytes primed against AAL display specific immunological memory to the original stimulator, and whether this response is inhibited by the mouse monoclonal antibody (MAb) to human Ia-like antigens, MAb Q5/13 (10). An example of the results obtained is given in Table I. Purified T lymphocytes from individual A, whose phenotype is DR3,DR5, were primed against AL, which had been alloactivated in a 5-d MLC with a DR2, DR5 stimulator (individual B). This PLT, primed to "autologous anti-DR2 blasts," was rechallenged with the following irradiated stimulators: (a) autologous anti-DR2 blasts derived from the same responder-stimulator combination (ABx) and from another MLC (AEx) in which the DR3,DR5 responder was primed against a DR2 homozygote (individual E); (b) autologous anti-DR4 blasts (derived from an MLC [ACx] with individual C, a DR3,DR4 stimulator); (c) autologous anti-DR1 blasts (derived from an MLC (ADx) with individual D, a DR1,DR3 stimulator); and (d) monocyte-depleted lymphocytes from each allostimulator (B, C, D, and E) used for priming the AL. These stimulators were also used for challenging a 9-d PLT raised by priming PBL from individual B against lymphoblasts derived from an A-anti-B MLC. Parallel cultures were tested under the same conditions, yet 20 μ l of MAb Q5/13 were added to each reaction at the time when the stimulators were plated.

T lymphocytes primed to autologous anti-DR2 blasts displayed secondary, accelerated responses only when rechallenged with AL activated against DR2 in MLC with the original DR2, DR5 stimulator (individual B) or in MLC with a DR2, DR2 homozygous donor (individual E). In contrast, AL primed against DR4 or DR1 failed to induce a secondary MLC response. Such anti-self-primed lymphocytes also failed to display an accelerated (66 h) response when challenged with autologous or allogeneic PBL. The monoclonal anti-Ia had no effect on the magnitude of these reactions. Fresh (resting) OKT-3⁺ lymphocytes from the same responder (A) displayed little, if any, reactivity to AAL and responded weakly to allogeneic stimulators, as expected, because cultures were harvested on day 3, rather than on day 6 when the peak of primary MLR occurs. The allogeneic MLR responses were almost obscured in the presence of MAb Q5/13.

Lymphocytes from individual B (DR2, DR5), primed against lymphoblasts from an A-anti-B MLC, behaved like an anti-DR3 PLT. They were reactivated by all the MLC-stimulated blasts derived from individual A (i.e., from the ABx, ACx, ADx, and AEx MLC) as well as by PBL from the DR3-positive (A, C, and D) but not from the DR3-negative (B and E) donors. Thus, as proven in this allogeneic PLT system, all MLC-alloactivated lymphoblasts from individual A were endowed with MLC

TABLE I
Secondary AMLR (3-d Culture)

Responders (OKT-3 ⁺ T cells)	Mono- clonal anti-Ia added	Irradiated stimulating cells								
		MLC-activated T lymphoblasts*				Monocyte-depleted PBL				
		ABx	ACx	ADx	AEx	Ax DR3,5	Bx DR2,5	Cx DR3,4	Dx DR1,3	Ex DR2,2
A primed to ABx	No	6,320	620	430	5,420	310	450	620	830	540
	Yes	5,840	780	340	6,210	230	260	580	1,010	860
A resting T cells‡	No	1,560	1,810	1,940	1,270	960	8,410	4,512	5,710	4,510
	Yes	960	1,200	1,660	540	410	3,200	2,100	2,350	1,240
B primed to ABx	No	15,230	21,710	11,210	13,840	19,420	1,210	17,800	12,210	2,210
	Yes	6,820	5,490	6,370	4,550	10,250	340	2,500	4,820	1,406
B resting T cells§	No	4,810	5,710	6,820	3,290	6,540	850	4,850	5,010	320
	Yes	1,240	2,260	2,410	1,820	3,770	680	2,110	1,980	640

Results are expressed as mean cpm of triplicate cultures.

* MLC-activated T lymphoblasts: ABx DR3,5 primed to DR2,5; ACx DR3,5 primed to DR3,4; ADx DR3,5 primed to DR1,3; AEx DR3,5 primed to DR2,2.

‡ Resting fresh T cells from individual A.

§ Resting fresh T cells from individual B.

stimulatory activity, although only lymphoblasts primed to DR2 reactivated the secondary AMLR in the autologous system. When the anti-DR3 PLT was tested in the presence of monoclonal anti-Ia antibody, a significant inhibition of secondary responses was observed. Fresh lymphocytes from individual B responded to all allogeneic stimulators in the expected pattern and showed decreased reactivity in the presence of anti-Ia antibody.

Results of three additional experiments confirming the specificity of the memory response to AAL are illustrated in Fig. 1. In experiment I, the responder (DR2, DRw6) was primed against AL that had been activated against DR5 (i.e., against stimulator B with the DR2, DR5 phenotype). This PLT was reactivated by AL sensitized either to the original stimulator (B) or to other DR5-positive individuals (C, D, and E), but not by AL sensitized to DR7 (F) or DR3 (G). In experiment II, the responder (DR2, DR5) was primed to AL that had been alloactivated against DR3 (present on stimulator B with the DR2, DR3 phenotype). Secondary responses were induced by AL activated against DR3, but not by AL activated against another DR antigen such as DRw6. In experiment III, a DR3, DR5 responder was primed to AL alloactivated against DR4. Like in the previous experiments, restimulation was induced by AL primed to the DR antigen of the original allostimulator (DR4 in this case).

In all three experiments, resting lymphocytes displayed a low 66-h response to AL, regardless of the DR specificity against which the AL were alloactivated. The significantly higher 66-h reactivity exhibited by the anti-AL PLT can therefore be attributed to accelerated, memory responses. In experiments I and II, the AMLR response to autologous phytohemagglutinin (PHA)-activated lymphoblasts was also tested. The reactivity displayed by anti-AAL-primed lymphocytes to autologous PHA-blasts was of the same order of magnitude as that displayed by resting OKT-3⁺ cells.

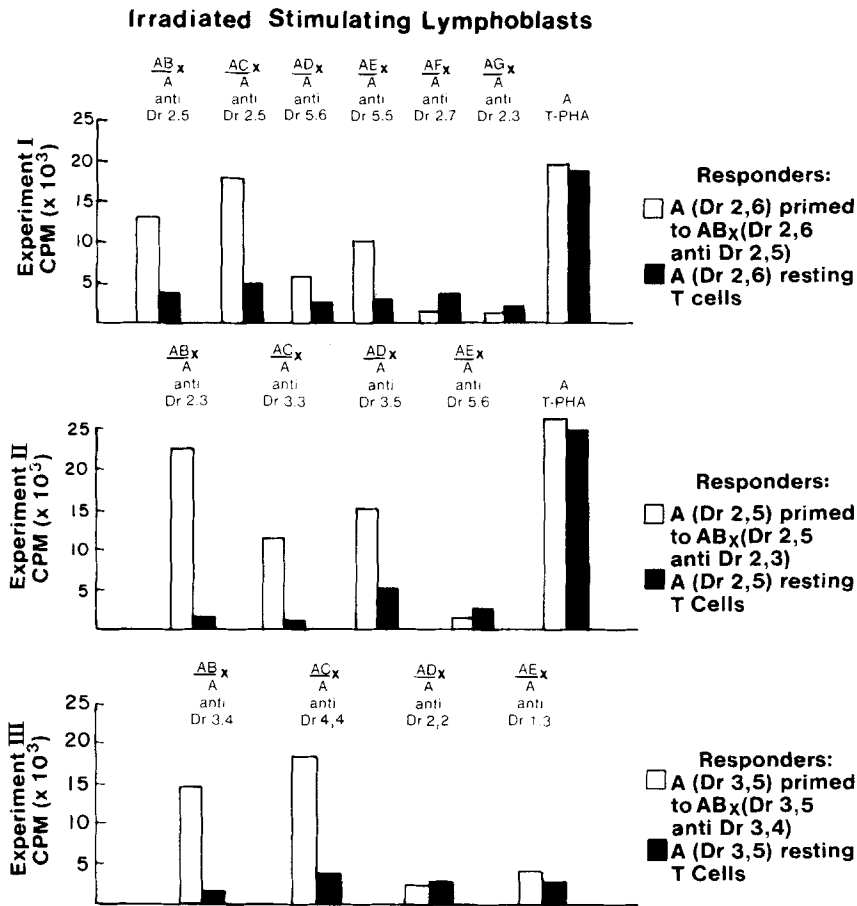


FIG. 1. Lymphocytes primed in primary AMLR to autologous alloactivated T lymphoblasts display secondary AMLR responses only when restimulated with autologous T lymphoblasts sensitized to the same HLA-DR alloantigen as the primary stimulators.

Discussion

Human T lymphocytes seem to have clonally distributed receptors for HLA-D/DR antigens as demonstrated by the specificity of the recognition and memory phase of the *in vitro* (MLC) response to allogeneic stimulators (11, 12). The present study suggests that T lymphoblasts alloactivated against a given HLA-DR antigen express idiotypic-like receptors, as they are capable of inducing an AMLR response specific for the receptor expressed by the AAL. Thus, PLT generated against AL primed to a certain allogeneic HLA-DR are restimulated only by AL sensitized to the same specificity and not by AL alloactivated against a different HLA-DR.

Because T lymphocytes may have on their surface not only antigen-binding idiotypic receptors but also alloantigens of the stimulator type, it could be argued that both the primary and the secondary AMLR were, in fact, triggered by such soluble HLA alloantigen(s) coating the outersurface of the MLC blasts. This does not seem to be the case, however, as allogeneic PBL from the sensitizing donor failed to induce an accelerated response when used for testing the specificity of the secondary AMLR.

On the other hand, because Ia antigens may account for the capacity of B cells and monocytes to stimulate in AMLR (4–6), the question might be raised whether the response induced by MLC alloactivated T cells is caused by their capacity to express HLA-D and DR antigens (1–3, 13, 14). This possibility is unlikely in view of two observations. First, AAL primed against HLA-DR antigens, different from that against which the primary (AAL) stimulator was sensitized, did not trigger the secondary PLT response. Second, the specific memory response displayed by such PLT was not inhibited by the monoclonal anti-Ia, as would have been expected if the response were directed against Ia.

Autologous stimulation of primed lymphocytes that does not require specifically stimulated cells, such as non-T cells or PHA-activated lymphocytes, has been described, however, by other authors (15–17). Although idiotype determinants expressed by B lymphocytes or by polyclonally PHA-activated T cells might also be involved in such secondary AMLR, the self-stimulatory capacity of IgM-negative B cells, monocytes, and null cells (4–6) calls for a different explanation. This might well reside in the possibility that the human AMLC response is primarily specific for the heterologous proteins adsorbed to antigen-presenting cells during rosette formation with SRBC (18). This phenomenon had no impact on our own experiments as the secondary AMLR response was specific for the idiotype-like determinant expressed by the primary stimulator.

Finally, the above considerations do not exclude the possibility that recognition of self-Ia is part of the process of self-non-self discrimination. In fact, previous studies showing that strong anti-allo-Ia reactive T cell receptors also exhibit weak reactivity to self-Ia may indicate that reactivity to self-Ia, plus additional antigen, represents the true biological reason for the existence of the large repertoire of alloreactive T cell clones (19).

Summary

T cells alloactivated in 5-d MLC with an HLA-DR-different stimulator acquire the capacity of stimulating the autologous mixed lymphocyte response (AMLR). We have demonstrated that activation of AMLR by allosensitized T cells is determined by the expression of the idiotype receptor for the stimulating HLA-DR alloantigen. This has been shown in experiments in which purified, OKT-3-positive T cell suspensions were first primed for 9 d with AMLR-activated T lymphoblasts, then tested in secondary AMLR with autologous lymphoblasts sensitized to various HLA-DR alloantigens. Accelerated memory responses were induced only by autologous lymphoblasts that had been sensitized against the same HLA-DR specificity as the primary AMLR stimulators. This response was not inhibited by a mouse monoclonal antibody recognizing Ia-like determinants, and was not triggered by human allogeneic resting peripheral blood lymphocytes. Thus, recognition of alloactivated T lymphoblasts in secondary AMLR seems to be specific for the idiotype-like determinants expressed by the autologous stimulators.

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