Brief Definitive Report

CELLULAR BASIS OF THE PROLIFERATIVE RESPONSE OF HUMAN T CELLS TO MOUSE XENOANTIGENS

By BARBARA J. ALTER AND FRITZ H. BACH

From the Immunobiology Research Center, Departments of Laboratory Medicine/Pathology and Surgery, University of Minnesota, Minneapolis, Minnesota 55455

In a human MLC, highly purified peripheral blood T cells proliferate in response to allogeneic peripheral blood stimulator cells (1). This implies that human alloantigens can be recognized "directly" on the allogeneic stimulator cells, i.e., without participation of autologous antigen presenting/processing cells (APC) (2–5). In contrast, in the xenogeneic model of a human anti-mouse MLC, purified human responder T cells are unable to proliferate to murine spleen cells; apparently human T cells do not respond to the mouse xenoantigens "directly" on the murine stimulator cells. When human APC are present in the xenogenic MLC (6, 7), a response does take place that is presumed to be an "indirect" response in which the xenogeneic antigens are presented as peptides on the responder (self) APC.

There are several possible explanations for the failure to find a "direct" human anti-mouse response, including (*a*) a failure of human accessory molecules (CD4, CD8, LFA1, CD2) to find their appropriate ligands on murine cells (8); (*b*) the failure of the human T cell repertoire to include direct recognition of xenogeneic antigens, presumably based on the fact that the human T cells educated in a human thymus are not selected to recognize a molecule as disparate as mouse H-2 antigens, or (*c*) that human T cells can recognize xenoantigen directly but that requisite cytokines produced by murine APCs, e.g. IL-1, are not available in a form and/or amount adequate to promote proliferation in human T cells.

We report here the finding that highly purified human T cells can respond directly and in an antigen-specific manner to murine xenoantigens in the presence of human recombinant IL-1, IL-2, or a T cell growth factor (TCGF) preparation. These findings demonstrate that the human T cell repertoire does include the ability to recognize very widely disparate, i.e., murine, xenoantigens and that direct recognition can be obtained provided human cytokines are included in the cell reaction mixture.

Materials and Methods

Cell Isolation–responding Cells. Human responding cells are either PBMC from the interface of a Ficoll-Hypaque gradient or highly purified T cells from peripheral blood. To obtain

This is Immunobiology Research Center Paper No. 517. This work was supported in part by National Institutes of Health grants AI-17687, AI-22682, and HD-19973 and by the Harry Kay Chair for Biomedical Research Endowment.

Address correspondence to Dr. Fritz H. Bach, University of Minnesota, Immunobiology Research Center, Department of Laboratory Medicine and Pathology, Box 724, 420 Delaware Street SE, Minneapolis, MN 55455.

purified T cells, PBMC are incubated on nylon wool columns in 10% human serum RPMI 1640 with 25 mM Hepes and antibiotics and L-glutamine, i.e., complete tissue culture media (TCM). After 60 min at 37°C, nonadherent cells are eluted and depleted of adherent cells by incubation in 100-mm petri dishes (No. 1029; Falcon Labware, Oxnard, CA) overnight at 37°C in 5% CO_2 and air. Any residual non-T cells are removed from the petri dish nonadherent fraction by antibody plus complement depletion as previously described (9), using anti-human HLA-DR (No. 7360; clone L243; Becton Dickinson [B-D] Mountain View, CA), anti-human Leu-7 (B-D No. 7390, clone HNK-1), anti-human Leu-11b (B-D No. 7530, clone GO22), anti-Mo2 (No. 660214; Coulter Immunology, Hialeah, FL), and anti-NKH-1A (No. 6603420; Coulter Immunology). Cells are incubated with these antibodies at 4°C for 45 min, centrifuged, and the cell pellet resuspended in Lympho-Kwik T (One Lambda, Inc., Los Angeles, CA) and incubated at 37°C for 30 min. After washing, these cells are referred to as purified T cells.

Stimulating Cells. Murine stimulating cells are spleen cells (depleted of RBC) from BALB/c, B10.BR, (Jackson Laboratories, Bar Harbor, ME) and B10.Q (from Dr. Chella David, Mayo Clinic, Rochester, MN). The cells are irradiated with 2,500 rad. Human stimulating cells are PBMC irradiated with 4,000 rad.

Cell Cultures. MLCs are composed of responding cells (10^5/well) mixed with murine stimulating cells (8–10 × 10^5/well). Human autologous PBMC, used as a source of reconstituting accessory cells or APC, are irradiated (4,000 rad) and used at 10^5/well. Human allogeneic, irradiated stimulating PBMC are used at 3 × 10^5 cells/well. Cultures are incubated in 5% CO_2 at 37°C in 200 μl TCM in 96-well, flat-bottomed Linbro plates (No. 76-032-05; Flow Laboratories, MacLean, VA) for 5 d and labeled with 1 μCi [^{3}H]TdR (6.7 Ci/mM) during the final 7 h of culture. Data are expressed as mean counts per minute (cpm) ± standard deviation (SD) of triplicate cultures.

Primed Lymphocyte Typing (PLT). For PLT cultures (6, 10), 10^7 human responding T cells or PBMC (as indicated) are cultured together with 1.9 × 10^7 BALB/c_x spleen cells in the presence of irradiated reconstituting autologous human PBMC at 10^7 per flask or cytokines (rIL-1, rIL-2, TCGF concentrations given in the text) in 20 ml TCM in upright 25-cm^2 Corning flasks (No. 25100; Corning, NY). After 3 d of culture, 10 ml fresh TCM is added and every 2–3 d thereafter 8 ml of medium is removed from each flask and 8 ml fresh TCM is added. After 10–11 d of culture, the cells are restimulated in PLT in TCM in flat-bottomed 96-well Linbro plates at 10^5 primed responding cells per well with 10^6 irradiated murine stimulating cells or 2 × 10^5 irradiated allogeneic human PBMC. Cultures are terminated 2, 3, or 4 d later. Cells are labeled with [^{3}H]TdR during the last 7 h of culture and data are expressed as noted above.

Cytokines. rIL-1β (Genzyme, Boston, MA) and rIL-2, a gift from Cetus Corp., (Emeryville, CA) are given in units per milliliter. TCGF is given as percent of final total volume in culture (Catalog No. 812810, Lymphocult-T; BioTest, Frankfurt, Federal Republic of Germany). OKT3 (Ortho Diagnostic Systems, Westwood, MA) is used at 50 ng/ml final concentration. PHA-P (No. L-9132, Sigma Chemical Co., St. Louis, MO) is used at 1% vol/vol final concentration in cultures.

Results and Discussion

Purified T cells used in these studies were assessed for functional purity by their inability to respond to OKT3 and a markedly reduced response to PHA (Table I). That the T cells were functionally intact was verified by their response to these stimuli if irradiated, autologous PBMC, as a source of APC, were present in the culture system (i.e., a "reconstituted response"). This "reconstituted" response to PHA or OKT3 was, in all cases, comparable to that of unseparated PBMC (Table I).

Results shown in Table II from three separate experiments confirm earlier findings that purified human T cells do not respond proliferatively to mouse xenoantigens (6, 7). Addition of autologous human PBMC (x-irradiated) as a source of APC to

TABLE I

Lack of Response of Highly Purified T Cells to Polyclonal Stimulation

Responder cell	Stimulus	Exp. 1003	Exp. 1005	Exp. 1006
			mean cpm ± SD	
A_{PBMC}	TCM	63 ± 8*	174 ± 124	165 ± 24
	PHA	144,791 ± 4,232	312,771 ± 6,720	443,677 ± 6,366
	OKT3	82,446 ± 10,625	164,331 ± 20,990	205,587 ± 11,858
A_T	TCM	29 ± 10	49 ± 3	52 ± 12
	PHA	488 ± 106	2,017 ± 210	4,648 ± 364
	OKT3	72 ± 25	68 ± 25	59 ± 12
A_T	TCM + A_{PBMC_x}	NT	NT	69 ± 17
	PHA + A_{PBMC_x}	NT	249,369 ± 10,883	403,306 ± 26,920
	OKT3 + A_{PBMC_x}	NT	150,264 ± 12,995	138,297 ± 12,688

the human T cell–mouse spleen cell mixture reconstitutes a strong proliferative response to the BALB/c xenoantigens. This T cell response is always greater, in terms of cpm incorporated, than the response of human PBMC to murine stimulating cells (data not shown). In contrast, human T cells cultured in MLC with allogeneic irradiated PBMC respond proliferatively, as has been shown previously (1), and do not require additional "reconstituting" cells.

To understand the mechanism(s) whereby irradiated autologous PBMC reconstitute a T cell xenogeneic response, we used human cytokines to substitute for the human PBMC/APC. Data in Table II show that addition of rIL-1, rIL-2, or TCGF to the purified T cells allows a significant proliferative response to murine stimulating cells. As shown, the purified T cells in the absence of BALB/c stimulating cells do not respond to rIL-1; the response to rIL-2 or TCGF alone is very weak compared with the response in the presence of both the cytokine and the BALB/c cells.

We then used a PLT approach (10) to evaluate the immunogenetic specificity of the human T cell proliferative response to BALB/c xenoantigens. Purified human T cells were cultured with BALB/c-irradiated splenic stimulating cells in the absence or presence of autologous, irradiated PBMC or rIL-1 or rIL-2 or TCGF. After 10 d of culture, the primed responding cells were restimulated with irradiated cells from several sources. Results are shown in Table III. T cells primed to BALB/c in the presence of $APBMC_x$ or rIL-1, or rIL-2, or TCGF show a highly significant secondary proliferative response to BALB/c restimulating cells as is expected if the primary proliferative response was specifically to BALB/c antigens; there is no proliferative response to stimulating cells of other mouse strains, $B10.Q_x$ or $B10.BR_x$. These results demonstrate that cells proliferating in the primary human anti-mouse MLC in the presence of rIL-1, rIL-2, or TCGF respond in an antigen-specific manner. The relatively low level proliferation seen on day 2 in response to allogeneic human cells ($BPBMC_x$) in the PLT probably reflects the onset of a primary allogeneic response of unprimed T cells present in the responding population. We cannot rule out shared specificities between mouse and human stimulating antigens that may also contribute to the allogeneic proliferative response.

The lack of any response to the third-party murine stimulator cells in the PLT

TABLE II

Primary MLC Response of Purified Human T Cells to Murine Xenoantigen

Exp.	Added stimulus	TCM	A_{PBMC_x}	rIL-1	rIL-2	TCGF	B_{PBMC_x}
				mean cpm ± SD			
1003*	TCM	62 ± 3	224 ± 220	56 ± 12	699 ± 208	4,737 ± 1,018	16,703 ± 4,943
	BALB/c_x	102 ± 28	15,678 ± 15,628	5,236 ± 3,361	11,516 ± 2,319	35,430 ± 8,738	NT
1005‡	TCM	184 ± 203	47 ± 22	47 ± 8	3,074 ± 323	1,880 ± 240	115,430 ± 9,348
	BALB/c_x	510 ± 319	36,322 ± 14,003	4,963 ± 1,349	23,098 ± 1,545	40,920 ± 3,604	NT
1006§	TCM	55 ± 7	46 ± 7	192 ± 298	2,449 ± 63	2,008 ± 127	106,426 ± 6,083
	BALB/c_x	336 ± 358	25,013 ± 8,770	10,197 ± 1,929	34,794 ± 10,214	40,709 ± 3,164	NT

* Exp. 1003: rIL-1, 10 U/ml; rIL-2, 10 μ/ml; TCGF, 10%.
‡ Exp. 1005: rIL-1, 10 U/ml; rIL-2, 40 μ/ml; TCGF, 10%.
§ Exp. 1006: rIL-1, 9 U/ml; rIL-2, 200 μ/ml; TCGF, 10%.
Purified T cells of a different individual were used as responding cells in each experiment.

TABLE III

PLT Response of Human T Cells Primed to Murine Xenoantigen

Primary stimulation day 0	Restimulus day 10	Proliferation following restimulation Day 2	Day 3	Day 4
A_T + BALB/c_x + TCM	TCM	129 ± 37	NT	NT
	A_{PBMC_x}	149 ± 12	77 ± 20	NT
	B_{PBMC_x}	522 ± 145	3,453 ± 680	NT
	BALB/c_x	219 ± 135	221 ± 118	NT
	B10.Q_x	190 ± 166	198 ± 58	NT
	B10.BR_x	97 ± 14	50 ± 8	NT
A_T + BALB/c_x + A_{PBMC_x}	TCM	154 ± 25	76 ± 57	62 ± 15
	A_{PBMC_x}	195 ± 46	93 ± 26	93 ± 33
	B_{PBMC_x}	1,166 ± 352	2,565 ± 799	13,377 ± 9,855
	BALB/c_x	4,090 ± 1,324	11,695 ± 5,538	3,275 ± 1,938
	B10.Q_x	1,362 ± 819	1,626 ± 780	2,016 ± 372
	B10.BR_x	1,547 ± 745	1,841 ± 1,302	961 ± 477
A_T + BALB/c_x + rIL-1 (10 U/ml)	TCM	154 ± 44	30 ± 2	50 ± 9
	A_{PBMC_x}	463 ± 443	98 ± 4	115 ± 21
	B_{PBMC_x}	8,141 ± 6,897	13,215 ± 2,623	52,266 ± 5,473
	BALB/c_x	62,055 ± 5,587	96,159 ± 11,000	23,942 ± 2,559
	B10.Q_x	881 ± 258	456 ± 46	972 ± 88
	B10.BR_x	631 ± 405	393 ± 199	207 ± 103
A_T + BALB/c_x + rIL-2 (10 U/ml)	TCM	215 ± 58	62 ± 26	54 ± 14
	A_{PBMC_x}	390 ± 70	189 ± 97	161 ± 3
	B_{PBMC_x}	8,747 ± 2,149	20,330 ± 4,993	63,257 ± 8,005
	BALB/c_x	29,632 ± 1,838	5,072 ± 733	737 ± 212
	B10.Q_x	1,278 ± 562	265 ± 95	735 ± 144
	B10.BR_x	1,772 ± 1,514	276 ± 234	166 ± 102
A_T + BALB/c_x + TCGF (10%)	TCM	137 ± 45	31 ± 2	62 ± 9
	A_{PBMC_x}	280 ± 145	177 ± 134	136 ± 63
	B_{PBMC_x}	9,701 ± 2,222	33,065 ± 4,871	91,774 ± 8,629
	BALB/c_x	39,850 ± 898	111,781 ± 12,610	72,412 ± 11,120
	B10.Q_x	1,904 ± 942	2,822 ± 827	1,986 ± 477
	B10.BR_x	513 ± 94	555 ± 483	499 ± 395

compared with the positive allogeneic response may be based on the need, as demonstrated in the primary response, for human cytokines to permit a human anti-mouse response. These data, in aggregate, suggest that the cytokine requirements differ for the primary and secondary xenogeneic responses.

It was clear from earlier results that human T cells can respond to murine antigens in the presence of human APC. Such a response could be based solely on recognition of murine peptides presented by those self-APC. The present results demonstrate that an antigen-specific human anti-mouse response based on direct recognition of the murine antigens in the presence of human cytokines does occur, i.e., that the human T cell repertoire includes receptors that permit direct recognition of murine antigens.

While the data shown here address the issue of the human T cell repertoire vis-a-vis recognition of highly disparate xenogeneic antigens, they do not provide direct information regarding the extent to which that repertoire may be quantitatively limited compared with the repertoire that recognizes alloantigens. The data also do not address the issue of the ability of human CD4 and CD8 molecules or of other human cell surface molecules to interact significantly with the murine homologues of their human ligands.

Our initial hypothesis for this study was that there is no response of purified human T cells to murine xenoantigens because there is a need for an APC-produced cytokine, such as IL-1, to allow the response to take place, and the murine IL-1 would not provide an adequate stimulus to the human cells under these conditions (6). Our finding that the addition of human rIL-1 to these cultures allowed a response suggests that this may be a reasonable explanation.

In the past we have presented evidence that the proliferative response in human anti-mouse MLC (in which both responder and stimulator APC were present) was very largely directed against class II antigens of H-2 (6). In that system, however, it was not clear whether the class II antigens were being recognized directly or indirectly; it is at least possible that the majority, or all, of that response was an indirect response in which murine class II peptides were presented on human, autologous APC. In the present studies, there is, by definition, direct recognition of the murine xenoantigens. Experiments are currently in progress to evaluate which class(es) of antigens are recognized and which subpopulations of human T cells are responding under these conditions.

Based on studies of alloantigen recognition, we proposed that stimulation of precursor T cells with alloantigen alone (signal 1 to the T cell) drove those cells along the pathway of maturation to become "poised" to receive help in the form of what is now recognized to be IL-2 by upregulating receptors for IL-2 (11). We would suggest from the data reported herein that direct recognition by human T cells of murine xenoantigens does occur, and results in the delivery of a signal 1 to the human T cells that then renders the T cells responsive to subsequent addition of rIL-1 (12) or rIL-2.

Summary

Purified human T cells respond proliferatively to allogeneic peripheral blood mononuclear (PBMC) stimulating cells but show no response to murine splenic stimulating cells. Two possible explanations for the lack of xenogeneic response are

that human T cells, educated in a human thymus, cannot directly recognize a molecule as disparate as mouse antigen encoded by H-2 and/or that a cytokine(s) produced by the APCs is needed to allow a proliferative response and that the cytokine(s) produced by murine APC do not provide an adequate stimulus to the human T cells under these conditions. We show here that highly purified human T cells can respond directly in an antigen-specific manner to murine stimulating cells if human rIL-1 or rIL-2 or a T cell growth factor (TCGF) preparation are present in the culture. These findings demonstrate that human T cells can recognize murine antigens and that a highly significant response can be obtained if a human cytokine is present to permit that response.

We thank Drs. Mark Benfield and Simon Panzer for valuable discussions during the progress of this work and Ms. Nancy Andresen and Ms. Kyle Walden for manuscript preparation. We also thank Ms. Genia Gordon and her staff for technical assistance.

Received for publication 19 September 1989 and in revised form 25 October 1989.

References

1. Alter, B. J., and F. H. Bach. 1970. Lymphocyte reactivity in vitro. I. Cellular reconstitution of purified lymphocytes. *Cell. Immunol.* I:207.
2. Kruisbeek, A. M., P. M. Andrysiak, and A. Singer. 1983. Self recognition of accessory cell Ia determinants is required for the in vitro generation of hapten-specific cytotoxic T lymphocyte responses. *J. Immunol.* 131:1650.
3. Singer, A., A. D. Kruisbeek, and P. M. Andrysiak. 1984. T cell-accessory cell interactions that initiate allospecific cytotoxic T lymphocyte responses: existence of both Ia-restricted and Ia-unrestricted cellular interaction pathways. *J. Immunol.* 132:2199.
4. Sprent, J., M. Schaefer, D. Lo, and R. Korngold. 1986. Properties of purified T cell subsets. II. In vivo responses to class I vs. class II H-2 differences. *J. Exp. Med.* 163:998.
5. Golding, H., and A. Singer. 1984. Role of accessory cell processing and presentation of shed H-2 alloantigens in allospecific cytotoxic T lymphocyte responses. *J. Immunol.* 133:597.
6. Lindahl, K. F., and F. H. Bach. 1976. Genetic and cellular aspects of xenogeneic mixed leukocyte culture reaction. *J. Exp. Med.* 144:305.
7. Brunswick, M., and P. Lake. 1986. Functional interactions of human and murine lymphoid cells. *Cell. Immunol.* 103:441.
8. Martz, E. 1987. LFA-1 and other accessory molecules functioning in adhesions of T and B lymphocytes. *Hum. Immunol.* 18:3.
9. Baroja, M. L., J. L. Ceuppens, J. Van Damme, and A. Billiau. 1988. Cooperation between an anti-T cell (anti-CD28) monoclonal antibody and monocyte-produced IL-6 in the induction of T cell responsiveness to IL-2. *J. Immunol.* 141:1502.
10. Sheehy, M. J., P. M. Sondel, M. L. Bach, R. Wank, and F. H. Bach. 1975. HL-A LD (lymphocyte defined) typing: a rapid assay with primed lymphocytes. *Science (Wash. DC).* 188:1308.
11. Bach, F. H., M. L. Bach, and P. M. Sondel. 1976. Differential function of major histocompatibility complex antigens in T lymphocyte activation. *Nature (Lond.).* 259:273.
12. Oppenheim, J. J., E. J. Kovacs, K. Matsushima, and S. K. Durum. 1986. There is more than one interleukin 1. *Immunol. Today.* 7:45.