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Research Article

Immune T cells proliferate in response to antigen that is recognized in association with self-Ia determinants. T cells from a patient with severe combined immunodeficiency that has been successfully reconstituted with haplotype-mismatched, maternal bone marrow were studied in an attempt to understand the development of Ia restriction of antigen recognition in man. All the patient's T cells were of maternal origin as determined by HLA typing. The patient received a series of three immunizations with tetanus toxoid (TT) antigen between the 6th and 14th week posttransplant. TT-specific T cell lines were established from the patient's peripheral blood at 6 and 8 mo posttransplantation and were maintained in culture in the presence of irradiated monocytes from the patient, TT antigen, and interleukin-2. HLA typing of the two T cell lines revealed them to be exclusively of donor origin. Both T cell lines could proliferate to TT in the presence of monocytes derived from either the patient's mother or father. In contrast, a TT-specific T cell line obtained from the patient's mother proliferated to TT in the presence of autologous monocytes, but not in the presence of monocytes derived from the patient's father. Studies using monocytes from a panel of HLA-typed donors indicated that the patient's T cell lines proliferated to TT in the presence of monocytes that expressed the paternal DR […]

Major Histocompatibility Restriction of Antigen Recognition by T Cells in a Recipient of Haplotype Mismatched Human Bone Marrow Transplantation

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ABSTRACT Immune T cells proliferate in response to antigen that is recognized in association with self-Ia determinants. T cells from a patient with severe combined immunodeficiency that has been successfully reconstituted with haplotype-mismatched, maternal bone marrow were studied in an attempt to understand the development of Ia restriction of antigen recognition in man. All the patient's T cells were of maternal origin as determined by HLA typing. The patient received a series of three immunizations with tetanus toxoid (TT) antigen between the 6th and 14th week posttransplant. TT-specific T cell lines were established from the patient's peripheral blood at 6 and 8 mo posttransplantation and were maintained in culture in the presence of irradiated monocytes from the patient, TT antigen, and interleukin-2. HLA typing of the two T cell lines revealed them to be exclusively of donor origin. Both T cell lines could proliferate to TT in the presence of monocytes derived from either the patient's mother or father. In contrast, a TT-specific T cell line obtained from the patient's mother proliferated to TT in the presence of autologous monocytes, but not in the presence of monocytes derived from the patient's father. Studies using monocytes from a panel of HLA-typed donors indicated that the patient's T cell lines proliferated to TT in the presence of monocytes that expressed the paternal DR antigen (HLA-DR4) inherited by the patient but not in the presence of monocytes that expressed the paternal DR antigen (HLA-DR1) not inherited by the patient or in the presence of monocytes bearing irrelevant DR antigens. Monocytes that expressed either one of the two maternal DR antigens (HLA-DR3 and DR5) could support the proliferation of the patient's T cell lines in response to TT antigen.

HLA typing of the patient's monocytes at 6 mo posttransplant revealed only recipient HLA-DR antigens (HLA-DR3 and DR4). At 12 mo posttransplant, the patient's monocytes expressed recipient HLA-DR antigens as well as the non-shared HLA-DR5 antigen of donor origin.

The results of the present study indicate that T cells of human bone marrow chimera recognized antigen in the context of Ia determinants of recipient origin. The apparent recognition of antigen by the chimera's T cells in the context of donor Ia determinants that were not shared with the recipient is discussed.

INTRODUCTION

It is now well established that T cells recognize antigen in association with major histocompatibility complex (MHC) determinants (1, 2). In the case of proliferating helper T cells, antigen recognition is restricted by products of the Ia region of the H2 complex in mice (3) and by products of the Ia region homologue, HLA-DR, in man (4). The development of Ia restriction of antigen recognition by T cells has been the subject of intensive investigation in experimental animals for several years (5–8). Experiments with bone marrow chimeras in which bone marrow cells are injected into irradiated semiallogeneic or fully allogeneic hosts have indicated that the chimera's T cells are of donor origin, but are restricted in their capacity to recognize antigen by Ia antigens of the recipient in which they mature; the thymus appears to play the major if not exclusive role in the determination of Ia restriction (9–11).

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To date, no experiments have been reported on the development of MHC restriction in T cells of human recipients of HLA-mismatched bone marrow. Recently, immunologic reconstitution with T cell-depleted, haplotype-mismatched, maternal bone marrow has been achieved in a patient with severe combined immunodeficiency (12). After transplant, T cells from this patient were found to be of donor origin and to be tolerant to recipient histocompatibility antigens. The ability of these T cells to respond to specific antigens provided a unique opportunity to study the MHC restriction of antigen recognition by T cells in a semiallogeneic, human bone marrow chimera.

METHODS

Patient. The clinical course of the patient before transplantation and in the immediate posttransplant period had been reported previously (12). The patient was given a series of three immunizations with tetanus toxoid (TT) and diphtheria toxoid (DT)[1] at 1-mo intervals which began at the sixth week posttransplant. After this series of immunizations, the patient exhibited a positive delayed hypersensitivity skin test to TT antigen and her peripheral blood mononuclear cells (PBMC) proliferated vigorously in vitro in response to stimulation with TT. The patient continued to do well in the 1 yr that has elapsed since the transplant.

Isolation of PBMC and of monocytes. PBMC were isolated from heparinized blood by Ficoll-Hypaque centrifugation. Monocytes were isolated by adherence to plastic plates, as in reference 13.

Reagents. TT and DT, that had been obtained from the Massachusetts Biological Laboratories, Boston, MA, were dialyzed extensively against 0.15 M saline and diluted to the appropriate concentration in RPMI 1640 medium (Microbiological Associates, Walkersville, MD) before use.

Interleukin-2 (IL-2) containing supernatants was generated as follows. PBMC, which were depleted of monocytes by adherence to plastic petri dishes overnight, were irradiated with 1,000 R and suspended at 1×10^6 cells/ml RPMI 1640 medium supplemented with 2% AB serum and containing 1% phytohemagglutinin-M (Difco Laboratories Inc., Detroit, MI). After a 48-h incubation, the cells were centrifuged at 200 *g* for 10 min and the supernatants were collected and passed through a 0.45-μm filter. Supernatants were stored at 4°C.

Establishment of TT-specific T cell lines. TT-specific T cell lines were established as described in reference 13 from PBMC isolated from the patient at 6 and 8 mo after successful bone marrow transplantation and from PBMC that were isolated from the donor after primary immunization with three doses of TT antigen. Briefly, PBMC were cultured at 10^6 cells/ml in RPMI 1640 medium with 10% AB+ serum (complete medium) and 20 μg TT/ml. 7 d later, the blasts were isolated by sedimentation in a multistep Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient, washed with Hanks' balanced salt solution (HBSS), and resuspended in culture at 10^5 cells/ml with 20% IL-2 containing supernatants and 80% fresh complete medium. A concentration of 20% IL-2 containing supernatants in fresh complete medium was found to be optimal for the growth of the T cells. Every 3 to 4 d, the cells were counted and diluted to a concentration of 10^5 cells/ml with 80% fresh complete medium and 20% IL-2 containing supernatants. Cell lines were restimulated with antigen (TT) and autologous-irradiated (5,000 rad) PBMC every 7–10 d.

Cell cultures. Cultures were performed in triplicate in flat-bottomed microculture plates (Linbro Scientific, Inc., Hamden, CT) in a volume of 0.2 ml complete culture medium. Cultures contained 2×10^4 T cell blasts, with or without 1×10^4 irradiated (5,000 rad) monocytes. TT and DT antigens were added at a concentration of 20 μg/ml, which was found previously to be optimal for proliferation. Incubation was carried out for 3 d. The cultures were then pulsed overnight with 0.8 μCi [*methyl*-^{3}H]thymidine (New England Nuclear, Boston, MA) in 20 μl and harvested in a multiple sample harvester (Skatron, A. S., Lierbyen, Norway). The radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, IL).

Surface markers. Cell surface markers were analyzed by indirect immunofluorescence using the monoclonal antibodies OKT3, OKT4, OKT8, OKM1, and anti-Ia (Ortho Pharmaceutical Co., Raritan, NJ) as described in reference 14 and using goat antisera to human immunoglobulins as in reference 15.

HLA-DR typing. HLA-DR typing of PBMC and of monocytes was performed by the laboratory of Dr. Ed Yunis by previously described cytotoxicity methods (16).

RESULTS

Specificity of the T cell lines to TT antigen. The specificity of the two T cell lines derived from the patient for TT antigen is shown in Table I. Neither of the two cell lines proliferated to TT antigen in the

TABLE I
Specificity of the T Cell Lines for TT Antigen

Stimulus*	cpm of [^{3}H]Thymidine incorporated per culture‡	
	T cell line no. 1 6 mo posttransplant	T cell line no. 2 8 mo posttransplant
—	283±27	429±26
IL-2 containing supernatants	63,399±3,438	78,420±4,269
TT	257±16	391±15
Autologous monocytes	316±31	464±24
Autologous monocytes + TT	58,963±2,817	77,208±3,492
Autologous monocytes + DT	342±20	430±38
Allogeneic pool of monocytes	585±43	818±51

* IL-2 containing supernatants was added at a concentration of 20%, TT and DT at a concentration of 20 μg/ml. Autologous monocytes and allogeneic monocytes were irradiated with 5,000 rad and incorporated <200 cpm [^{3}H]thymidine cultured alone or with TT.
‡ Values represent mean±SD of triplicate cultures. Similar results were obtained in two other experiments.

[1] *Abbreviations used in this paper:* DT, diphtheria toxoid; IL-2, interleukin-2; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; TT, tetanus toxoid.

absence of autologous monocytes, although they proliferated vigorously in response to IL-2 containing supernatants. In the presence of autologous irradiated monocytes, both T cell lines proliferated vigorously in response to TT antigen, but did not proliferate in response to diphtheria antigen to which the patient was immune. There was no reactivity of the T cell lines in response to a pool of irradiated allogeneic PBMC obtained from five normal subjects.

Analysis of the surface markers and HLA antigens of the two cell lines revealed that they were T3+ (>95%), T4+ (>95%), and Ia (>90%); all were exclusively of maternal origin. Since identical results were obtained with both cell lines, we will subsequently present data on only one of them.

MHC restriction of TT-specific T cells. Table II examines the capacity of TT-specific T cells derived from the recipient and from her mother to recognize TT antigen presented by monocytes derived from the patient and her parents. Maternal T cells residing in the recipient recognized TT antigen presented by monocytes from both the father and the mother (Table II, lines 2 and 3), whereas maternal T cells residing in the mother recognized TT antigen presented by maternal monocytes but not by paternal monocytes (Table II, lines 5 and 6). Monocytes derived from the recipient presented TT antigen to both maternal and recipient T cells (Table II, lines 1 and 4).

When considered with our previous demonstration that human T cells recognize TT in association with DR (or DR-linked) determinants (10), the above results suggested that the patient's T cells recognized TT in association with maternal as well as paternal DR (or DR-linked) determinants. Therefore, we examined the capacity of antigen-presenting cells obtained from a large panel of HLA-DR typed individuals to support the proliferation of the patient's T cell lines in response to TT. From the results shown in Table III, it appears that the patient's T cell lines recognized TT in association with either one of the two maternally derived DR determinants, HLA-DR3 and HLA-DR5. The patient's T cell lines recognized TT in association with the HLA-DR4 determinant that was shared between the father and the patient, but not in association with the HLA-DR1 determinant that was present in the father but not shared with the patient. T cells from a normal individual of HLA-DR type DR 1, 7 recognized TT antigen in association with only two HLA-DR determinants, namely DR 1 and 7, which were inherited from his mother and father, respectively.

HLA-DR antigens of the patient's monocytes. Prior to transplant, all the patient's monocytes were of recipient origin and were positive for HLA-DR3 and DR4. At 6 mo after transplant, the only detectable HLA-DR antigens were the same as those present pretransplant, i.e., recipient antigens. At 12 mo posttransplant, the HLA-DR antigens expressed by the patient's monocytes included recipient HLA-DR antigens (HLA-DR3 and DR5) as well as the nonshared HLA-DR5 antigen of donor origin (Table IV).

DISCUSSION

The results of the present study indicate that T cells of donor origin, which are present in the circulation of a recipient who received haplotype-mismatched maternal bone marrow, appear to recognize TT antigen in association with DR (or DR-linked) determinants of both donor and recipient origin. The capacity of the chimera's T cells to recognize antigen in association with MHC antigens of the recipient is clearly demonstrated by the observation that maternal T cells residing in the recipient, but not maternal T cells residing in the mother, proliferated in response to TT antigen presented by paternal monocytes (Table II). This could not be due to back stimulation of the T cells by soluble factors that were produced by irradiated cells from the father because purified monocytes and

TABLE II
Antigen Presentation to TT-Specific T Cell Lines

Responding cells*	Antigen-presenting monocytes	cpm of [^{3}H]Thymidine incorporated‡	
		Medium	TT
Maternal T cells in recipient	Patient (F_1 recipient)	468±29	58,327±3,175
	Mother (donor)	270±14	42,814±2,066
	Father	235±17	17,031±934
Maternal T cells in mother	Patient (F_1)	872±94	32,551±674
	Mother	446±89	50,380±1,695
	Father	1,093±141	1,847±202

* The experimental conditions are similar to those described in Table I.
‡ Values represent mean±SD. Similar results were obtained in two other experiments.

TABLE III
HLA-DR Restriction of Antigen Presentation to TT Specific T Cell Line

HLA-DR type of antigen-presenting cells‡	cpm of [^{3}H]Thymidine incorporated by T cell line from:§					
	Patient			Normal (DR 1,7)		
	Medium	TT		Medium	TT	
[3,4*]	468	85,353	84,884	—	—	—
[3,5]	270	70,140	69,870	—	—	—
[1,4]	235	19,283	19,048	—	—	—
1,5	612	26,235	25,623	947	18,631	17,684
3,-	947	56,481	55,534	820	1,043	223
1,7	294	356	62	921	28,764	27,843
2,-	142	228	86	739	964	225
2,8	309	441	132	685	1,183	498
3,4	—	—	—	916	1,348	432
5,6	587	31,752	31,165	696	472	−224
[4,7]	805	23,611	22,806	748	24,160	23,412
[1,3]	—	—	—	862	15,357	15,271
[1,7*]	—	—	—	548	37,069	37,015

‡ The asterisks refer to the patient and to the subject who was the source of the normal T cell line. The family of the patient and that of a normal HLA-DR 1,7 donor are marked by boxes. Culture conditions are as described in Methods and in Table I.
§ Values represent means of triplicate cultures. SD are omitted for the sake of clarity.

not PBMC were used as antigen-presenting cells and because the father was not immune to TT antigen. This conclusion was supported by the observation that allogeneic monocytes that bear the HLA-DR4 antigen shared between the father and the recipient also presented antigen to the patient's T cells (Table III).

The demonstration that maternally derived T cells residing in the recipient 6 to 8 mo posttransplant recognized antigen in association with the paternally derived HLA-DR4 (or HLA-DR4-linked) determinant of the recipient has several implications. First, it demonstrates that in man, as in mice, "self"-Ia recognition is independent of the T cell MHC genotype and is influenced by the environment in which the T cell matures (5, 7, 8, 11). Animal experiments indicate that a radioresistant host element dictates the self-Ia specificity of T helper cells (11, 17). The major site for T cell education in self-recognition appears to be thymus, although extrathymic sites may play a role in this process (18, 19). Second, the presence in the patient's T cell line of maternally derived T cells that recognized TT in the context of paternally derived HLA-DR4 suggested that, at the time of the study, the chimera possessed in her circulation monocytes of recipient origin that bore the HLA-DR4 antigen. Indeed, in the absence of such monocytes, HLA-DR4-restricted T cells could not have been propagated in culture. The presence of HLA-DR4+ monocytes in the patient's circulation was directly demonstrated by tissue typing 6 and 12 mo after transplant (Table IV). In contrast, as early as 3 mo posttransplant, all the patient's erythrocytes and neutrophils as well as her T cells were solely of maternal origin (12). Thus, the cytotoxic regimen used to prepare the recipient for transplantation (rabbit antilymphocyte serum, cyclophosphamide, and busulfan) did not result in the rapid elimination of cells of monocytic lineage. The relative resistance of cells of monocyte/macrophage to irradiation and their slow turnover is well documented in experimental animals (5–7, 17, 20).

TABLE IV
DR Type of Monocytes

Subject	DR type
Father	DR1, DR4
Mother	DR3, DR5
Patient pretransplant	DR3, DR4
6 mo posttransplant	DR3 (80%), DR4 (60%)
12 mo posttransplant	DR3 (80%), DR4 (40–50%), DR5 (30–40%)

Numbers in parentheses show percent cytotoxicity. The range refers to the percent cytotoxicity using different alloantisera to the particular specificity. Values of <25% were scored as negative.

Third, T cells from the chimera were previously shown to be unreactive in mixed lymphocyte culture to the recipient's cells taken prior to transplant (12). This unresponsiveness to paternally derived HLA antigens was not due to the presence of suppressor cells; it probably reflected the tolerance of maternal T cells maturing in the recipient for MHC antigens that were shared between recipient and father. The presence in the recipient of T cells that recognize antigen in association with the paternally derived HLA-DR4 (or HLA-DR4-linked) determinant to which they are tolerant suggests that in man, as in experimental animals, "self" tolerance and the ability to recognize self plus antigen develop together and probably involve the same mechanisms (21). These mechanisms are thought to involve the loss of high affinity, self-reactive cells and the retention and subsequent release into the periphery of low affinity, self-reactive cells that cross-react with self plus antigen (22).

The unexpected finding in our study was the observation that the chimera's T cell lines appeared to recognize TT in association with the HLA-DR5 antigen, which was present in the mother but not in the recipient. This is contrary to a vast body of evidence in experimental animals which suggests that antigen recognition by helper T cells of bone marrow chimeras is restricted by Ia antigens of recipient, but not of donor, origin (6, 11, 17). A number of explanations may have accounted for the apparent ability of the chimera's T cells to recognize TT in association with the HLA-DR5 antigen present in the donor, but not in the recipient.

First, it was possible that residual mature T cells that have already learned to recognize HLA-DR5 as self were transferred in the three bone marrow inocula that this patient received from her mother. In the initial two unsuccessful attempts at reconstituting the patient, mature T cells may have been transferred, but not in numbers sufficient to cause an acute graft-vs.-host disease. Although the third bone marrow inoculum contained <0.1% T3+ cells and failed to respond to phytohemagglutinin, it still contained enough mature functional T cells to cause a moderately severe acute graft-vs.-host disease which was terminated by in vivo infusion of monoclonal anti-T12 antibody (12). The possibility that transferred, mature donor T cells persisted in the patient's circulation in substantial numbers is made less likely by the failure of the chimera's peripheral blood lymphocytes to proliferate in response to recipient alloantigens that were expressed on mononuclear cells collected prior to bone marrow transplantation.

A second explanation for our unexpected finding is that the T cells recognize TT antigen not in association with HLA-DR, but in association with I region gene product(s) which differ from HLA-DR. In this case, such I region gene products could be shared between the recipient and the two HLA-DR5 positive subjects whose monocytes presented TT antigen to the chimera's T cells. Recent data from various laboratories including ours strongly suggest that antigen recognition by human T cells is indeed restricted by I region gene products which differ from HLA-DR (23, 24).[2]

A final explanation for our unexpected finding is that clones within the TT-specific T cell line were recognizing TT in association with a determinant shared between HLA-DR5 and either HLA-DR3 or HLA-DR4. Recognition of antigen by an antigen-specific T cell hybridoma in association with different I-A antigens has been recently demonstrated (25).

Whatever may be the basis for the recognition of TT in the context of HLA-DR5, this does not deny the importance of our major observation that donor T cells can cooperate with I region antigens of the recipient.

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