ANTIGEN RECOGNITION BY MHC-INCOMPATIBLE CELLS OF A HUMAN MISMATCHED CHIMERA

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T cells recognize antigen (Ag)[1] in association with MHC determinants (1). In most cases, helper/inducer T cells recognize Ag in the context of class II MHC molecules, while cytotoxic/suppressor cells recognize Ag in the context of class I MHC molecules. Although this concept of MHC restriction is well established, the mechanisms underlying its acquisition still remain unclear. To elucidate this point, numerous studies have been performed in murine lymphohaematopoietic chimeras (1–5). Experiments in which bone marrow or fetal liver cells are injected into irradiated semiallogeneic or fully allogeneic hosts have indicated that the chimeric T cells of donor origin are restricted in their capacity to recognize antigen by class II MHC molecules of the recipient in which they mature. Based on these observations, it has been suggested that MHC restriction is acquired during T cell ontogeny and is not genetically determined. The thymus appears to play the major role in the determination of this restriction (2, 6).

In contrast to animal models, studies on the development of MHC restriction by human T cells are very limited. Thus far, the majority of the results reported in the literature have been obtained in patients suffering from severe combined immunodeficiency (SCID) transplanted with HLA haploidentical bone marrow (7, 8). In this situation, contribution of the shared HLA haplotype to the development of T cell repertoire could not be ruled out. Another major drawback until now was that the in vitro experiments were not performed at the clonal level, therefore, the possibility of contamination by donor T cells in the host population and vice versa could not be excluded (7, 8).

Recently, we described patients suffering from SCID who were immunologically reconstituted after complete allogeneic fetal liver and thymus transplantations (FLTT) (9, 10). A more detailed study carried out on one patient, who now has a follow-up of 11 yr, showed that 7 yr after transplantation all T cells of the patient were of donor origin, whereas the B lymphocytes and monocytes were of host origin (10, 11). Despite this complete HLA mismatch between the fetus-derived T cells and the recipient cells, the patient had normal immune responses against pathogenic microor-

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[1] *Abbreviations used in this paper:* Ab, antibody; Ag, antigen; FLTT, fetal liver and thymus transplantation; SCID, severe combined immunodeficiency; TT, tetanus toxin.

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ganisms and thymus-dependent antigens (9, 11). Therefore, this patient represents a unique model to study the MHC restriction of Ag recognition by T cells. For this reason, tetanus toxin (TT)-specific T cell clones from the patient's PBL were established. In the present paper, we show that T cell clones of donor origin could specifically recognize TT in the context of recipient MHC resulting in proliferation. To our knowledge, this is the first demonstration that Ag recognition can occur in the context of "allo" MHC instead of "self" MHC at the clonal level in humans. In addition, TT-specific T cell clones able to recognize Ag in a non-MHC-restricted manner have been isolated.

Materials and Methods

Patient. The patient S.P.S. had a family history of SCID and was brought up in strict isolation from birth. At the age of 1 and 5 mo, he received FLTT from two different HLA-incompatible donors who were 13 and 10 wk old (gestational age), respectively. Only after the second transplant were clinical improvement and progressive immunological reconstitution observed. The clinical course of the patient before and after transplantations has been reported previously (9, 10). 2 yr after transplantation, the patient was immunized with TT by three injections at 1-mo intervals and serum anti-TT antibodies (Abs) were detected. The booster immunizations given in the following years always resulted in increased serum levels of anti-TT Abs. Anti-TT Ab production by PBL of the patient has been also demonstrated in vitro (11). In addition, the patient exhibited a positive delayed hypersensitivity skin reaction to TT.

The patient, who is 11 yr old now, has continued to do well since the transplantation, is in apparent good health, and is free of significant infections. Despite normal total lymphocyte counts and normal numbers of $CD3^+$ $CD2^+$ T cells, he has a persistent inverted $CD4^+/CD8^+$ ratio (ranging from 0.56 to 0.78). This inverted ratio is due both to a decrease in $CD4^+$ T cells and to an increase in $CD8^+$ T cells.

Preparation of TT-specific T Cell Lines and Establishment of T Cell Clones. Peripheral blood of the patient was drawn 2 wk after an in vivo immunization with TT. PBL were isolated by Ficoll/Hypaque density gradient centrifugation. 10^6 PBL were resuspended in 1 ml Yssel's medium (12) supplemented with 1% human AB^+ serum (heat inactivated, 30 min at 56°C) and were stimulated with TT (Calbiochem-Behring Corp., La Jolla, CA) at a concentration of 25 μg/ml. After incubation at 37°C in 5% CO_2 for 6 d, these TT-activated cells were resuspended in medium containing 20 IU rIL-2/ml (kindly provided by Dr. R. Kastelein, DNAX Research Institute, Palo Alto, CA). 12 d after the onset of the culture, the cells were cloned by limiting dilution at a concentration of one cell per three wells in 96-well round-bottomed plates (Titertek, Flow Laboratories, Irvine, Scotland) in the presence of a feeder cell mixture consisting of 5×10^5 irradiated (4,000 rad) allogeneic PBL per ml, 5×10^4 irradiated (5,000 rad) cells per ml of the patient's EBV-transformed B cell line (SPS), 0.1 μg/ml purified PHA (Wellcome Diagnostics, Beckenham, UK), and 25 μg/ml TT. After 14 d, proliferating T cell cultures were transferred to 24-well tissue culture plates (Linbro; Flow Laboratories) and restimulated with the feeder cell mixture. The clones were further expanded in medium containing rIL-2. 10 d after the last stimulation, the clones were screened for their specificity and functional activities as described below.

Cell Lines. The EBV cell lines of the patient (SPS), his father (UD93), and his mother (UD94) originated from infection of fresh PBL with EBV obtained from the marmoset lymphoblastoid cell line B 95-8. All cell lines were cultured in Yssel's medium supplemented with 1% human AB^+ serum.

Preparation of APC. The EBV cell lines and fresh PBL used as APC were irradiated at 5,000 and 4,000 rad, respectively, washed, and added to the culture.

In some proliferation experiments EBV cell lines or PBL were preincubated in the presence of 25 μg/ml of TT in 5% CO_2 at 37°C. After a period of 16–24 h, Ag-pulsed cells were washed three times, resuspended in complete medium, and used for Ag presentation.

For treatment of EBV cell lines with chloroquine (Sigma Chemical Co., St. Louis, MO),

cells were suspended at 10^6/ml in Yssel's medium and freshly prepared chloroquine was added at a final concentration of 0.1 mM 30 min before the addition of TT. After 5 h, the cells were washed three times with PBS and fixed with 0.05% glutaraldehyde for 1 min at room temperature. The reaction was stopped with 0.2 M glycine in PBS. The cells were washed three times and resuspended in complete medium.

The type I human T leukemia virus (HTLV-1)-transformed T cell clones B21 p19 and 827 p19 were obtained by cocultivating the TT-specific T cell clones B21, which is derived from the patient, and 827, which is of allogeneic origin, with the HTLV-1-secreting cell line C 91/pl (13). It has been shown recently that these HTLV-I-infected T cell clones have the ability to present antigen to MHC-restricted T cells (Yssel, H., R. de Wall Malefyt, M. Duc Dodon, D. Blanchard, L. Gazzolo, J. E. De Vries, and H. Spits, submitted for publication).

HLA Typing. HLA typing was carried out on the T cell clones and EBV-transformed B cell lines using a cytotoxicity assay as described previously (14).

Antigen-induced T Cell Proliferation. 9–12 d after stimulation with feeder cells, the cloned T cells were washed three times and 2×10^4 of these cells were incubated with 2×10^4 irradiated (5,000 rad) APC in the presence or absence of soluble TT at a final dilution of 1:100 corresponding to 25 μg/ml, or 10 μg/ml of purified protein derivative (PPD; a kind gift of Dr. Kreeftenberg, Rijks Instuut voor de Volksgezondheid, Bilthoven, Netherlands) in a final volume of 200 μl Yssel's medium with 1% human AB^+ serum. After 3 d of incubation, 1 μCi [^{3}H]TdR (New England Nuclear, Dreieich, Federal Republic of Germany) was added to each well. 4 h later, the cells were harvested onto glass fiber strips using a semi-automated cell harvester and the amount of incorporated [^{3}H]TdR was assessed by liquid scintillation counting. The results are expressed as the mean of triplicate cultures ± SD. The effect of mAbs on the proliferative capacity of T cell clones was determined by adding varied amounts of mAb at the onset of the cultures.

mAbs. The anti-CD2 mAb used in this study was CLB-T11 (kindly provided by Dr. R. Van Lier, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Netherlands). The anti-CD4 mAb RIV6 was kindly provided by Dr. Kreeftenberg (Rijks Instuut voor de Volksgezondheid, Bilthoven, Netherlands). The anti-CD8 mAb SPV-T8 and the anti-CD3 mAb SPV-T3b have been described before (15). The mAb W6/32, which detects a common determinant on all class I HLA molecules, was obtained from Sera Lab (Crawley Down, UK). The antibody SPV-L3 reacts with a monomorphic determinant on HLA-DQ molecules (15). The mAb Q5/13 was a kind gift from Dr. S. Ferrone (Medical College, Valhalla, NY) and detects a determinant common to HLA-DR and HLA-DP molecules (16). The mAb 135 detects a determinant on HLA-DR (17).

Fluorescence Analysis. 10^5 cells were added per well of a V-bottomed microtiter plate and washed once with PBS containing 0.02 mM NaN_3 and 1% BSA. The cells were then incubated with mAbs for 30 min at 4°C. After two washes in PBS/azide/BSA, the cells were incubated with a 1:40 dilution of FITC-labeled $F(ab')_2$ fragments of a goat anti-mouse antibody (Bioart, Meudon, France) and incubated for 30 min at 4°C. After three washes, the cells were transferred to FACS tubes (Becton Dickinson & Co., Oxnard, CA) and analyzed on a FACS (model 440; Becton Dickinson & Co.).

Results

HLA Typing of PBL, Cell Lines, and T Cell Clones. The HLA typing of parental PBL and patient's PBL before transplantation is shown in Table I. HLA typing of the patient's PBL, carried out by conventional cytotoxicity assays during the follow-up after transplant, revealed the progressive engraftment of cells from the second donor (HLA-A1,2; C7; B8,18; DR3,9) while the cells from the first donor (HLA-A2,11; C4; B62,27; DR1,8), present at low numbers at the beginning, progressively became less detectable in peripheral blood. Furthermore, the HLA typing, performed 7 yr after transplantation on separated T and B cell populations and monocytes (Table I) indicated that all the B lymphocytes and monocytes remained of host origin, whereas the T lymphocytes were donor derived.

TABLE I

HLA Typing of the Patient and his Parents

Subject	HLA locus A	HLA locus C	HLA locus B	HLA locus DR
Father*				
a	1	W4	17	7
b	W33	W6	14	4
Mother*				
c	3	W2	W47	5
d	W26	W4	12	1
Patient*				
b	W33	W6	14	4
c	3	W2	W47	5
Patient‡				
T cells donor	1-2	W7	8-18	3-W9
B cells host	W33-3	W6	14-W47	4-5
Monocytes host	W33-3		14-W47	4-5

* HLA genotypes of the family.

‡ HLA phenotype of the patient 7 yr after the transplantation. HLA typing was carried out on enriched cell populations.

HLA typing of the TT-specific T cell clones and the EBV cell line obtained from the patient's PBL (Table II) confirmed the split chimerism. The EBV cell line SPS has the HLA phenotype of the recipient, whereas the T cell clones have the HLA phenotype of the transplanted cells. As expected from the results obtained with PBL, the T cell clones F1, F9, F15, and F17 have the HLA phenotype of the second donor. Interestingly, T cell clones F3, F11, F13, and F14 have the phenotype of the first donor, indicating that, although these cells were not readily detectable in conventional HLA typing tests carried out on fresh PBL, they are present and functional.

Screening and Characterization of T Cell Clones. Screening of the T cell clones showed that 12 of 15 clones proliferated specifically in response to TT processed and presented by the patient EBV cell line SPS. The majority of these clones were also cytotoxic for SPS pulsed with TT (not shown). The responses were specific for TT, since none of these T cell clones could be induced to proliferate or were cytotoxic against SPS in the absence of TT. In addition, no responsiveness was observed in the presence of PPD (not shown). Analysis of the surface markers revealed that all the TT-specific cell clones were $CD2^+$, $CD3^+$, $CD4^+$, $CD8^-$.

TABLE II

HLA Typing of Patient's EBV Cell Line and T Cell Clones

	HLA				
Cell lines	A	C	B	DR	DQ
EBV cell line SPS (Recipient)	W33-3	W6	14-W47	4-5	W3
T cell clones					
F3, F11, F13, and F14 (1° FLTT)	2-11	W4	62-27	1-W8	W1
T cell clones					
F1, F9, F15, and F17 (2° FLTT)	1-2	W7	8-18	3-W9	W3

Clones F3, F11, F13, and F14, which have the HLA phenotype of the first transplant, and clones F1, F9, F15, and F17, which derived from the second donor, were selected for further characterization.

MHC Restriction of TT-specific T Cell Clones. To demonstrate that presentation of TT to the specific T cell clones could also be mediated by APC present in vivo, we examined the proliferative responses of T cell clones to TT using patient's and parents' fresh PBL as APC. As shown in Table III, all eight T cell clones recognized antigen presented by the fresh PBL of the patient, which contain both B cells and monocytes of recipient origin. In addition, T cell clones F9, F15, and F17 recognize TT presented by PBL of the mother, while T cell clones F11 and F13 recognize TT presented by PBL of the father. These data indicate that both the TT-specific T cell clones F9, F15, and F17, derived from the T cells of the second transplant, and the T cell clones F11 and F13, derived from the first transplant, are restricted by the HLA determinants of the recipient's fresh PBL.

Surprisingly, T cell clones F1, F3, and F14 proliferated to TT when both PBL of the father or the mother (which have no serologically defined HLA antigens in common in the haplotype inherited by the patient) were used as APC. This suggested that these clones either recognize TT in a non-MHC-restricted manner or recognize TT in the context of an unknown HLA epitope shared by the parents.

Analysis of Recipient MHC-restricted T Cell Clones. To further analyze the restriction element of T cell clones F9, F11, F13, F15, and F17, we examined the proliferative responses to TT using the parental EBV cell lines and a limited panel of allogeneic EBV cell lines as APC.

In Table IV, it is shown that T cell clones F9, F15, and F17 (which express HLA-DR3,9) recognized TT processed and presented by the mother's EBV cell line UD94 and by the allogeneic EBV cell line 11.3, which share HLA-DR5 antigen with the patient. The T cell clones F9, F15, and F17 failed to respond to TT plus the father's EBV cell line UD93 and JY, which share HLA-DR4 antigen with the patient, or the allogeneic EBV cell line NOB. In addition, when TT was presented by the EBV

TABLE III

Proliferative Response of T Cell Clones to TT Presented by Fresh PBL

		Responder T Cell Clones*							
APC	Ag	F9	F11	F13	F15	F17	F1	F3	F14
		cpm × 10^{-3}							
PBL									
Patient	-	0‡	0	0	0.3	0.6	0.8	0	0.1
Patient	TT	6.5§	17.0	37.9	11.5	31.4	28.2	10.4	3.9
Mother	-	0	0	0.4	0.5	0.1	0.1	0.1	0.1
Mother	TT	5.8	1.2	1.4	11.2	28.6	18.9	7.1	2.8
Father	-	0	0.1	0.4	0.2	0.4	0.2	0.1	0.1
Father	TT	0.3	14.5	28.8	1.7	1.1	13.5	7.4	3.5

* In all cases, the SD was <10% of the total cpm and the proliferation of T cells to TT in the absence of APC was <0.3 × 10^3 cpm.

‡ [^{3}H]TdR incorporation.

§ Significant [^{3}H]TdR incorporation is underscored.

TABLE IV

Proliferative Response of HLA Class II-restricted T Cell Clones to TT Presented by a Panel of EBV Cell Lines

APC	HLA-DR phenotype	Ag	Responder T cell clones*				
			F9	F11	F13	F15	F17
			cpm × 10^{-3}				
SPS	4,5	-	0.3‡	0.2	0.4	0.2	0.2
(Patient)		TT	26.6§	33.3	43.8	47.4	163.7
UD94	1,5	-	0.1	1.4	0.5	0.6	0.5
(Mother)		TT	11.0	1.3	0.8	5.6	5.4
UD93	7,4	-	0.1	0.5	0.4	0.5	1.6
(Father)		TT	0.3	30.1	35.9	2.3	3.0
NOB	2	-	0.1	0.3	0.1	0.6	0.2
		TT	0.6	0.9	1.5	1.5	0.4
JY	6,4	-	0.8	0.1	0.1	0.1	0.4
		TT	0.3	16.4	6.9	0.6	0.9
11.3	3,5	-	0.1	0.5	0.1	0.3	0.1
		TT	109.0	0.9	0.4	13.7	222.5
HSY	3,7	-	0.1	0.1	0.1	0.1	0.6
		TT	0.4	0.1	0.4	0.1	0.5
QBL	3	-	0.1	0	0	0	0
		TT	0.1	0	0	0.3	0
AVL	3	-	0.3	0	0	0	0
		TT	0.7	0	0	0.3	0.1

* In all cases, [^{3}H]TdR incorporation of T cells in the presence of TT alone was <0.4 × 10^3 cpm and the SD was <10%.

‡ [^{3}H]TdR incorporation.

§ Significant [^{3}H]TdR incorporation is underscored.

cell lines HSY, QBL, and AVL, which share HLA-DR3 with the T cell clones, no proliferative responses were detected. The other two T cell clones, F11 and F13 (HLA-DR1,8), recognized TT presented by the EBV cell line UD93 derived from the father and by JY, which expresses HLA-DR4. Taken together these results suggest that HLA-DR5 is the element of restriction of T cell clones F9, F15, and F17, while T cell clones F11 and F13 are restricted by HLA-DR4.

To confirm these data, blocking studies with mAbs directed against class I and II HLA antigens were performed. The proliferation of all five $CD4^+$ T cell clones, F9, F11, F13, F15, and F17, in response to TT presented by the recipient EBV cell line SPS, was strongly blocked by the anti-HLA-DR mAb 135 and by the anti-HLA-DR, DP mAb Q5/13. In contrast, mAbs against HLA-DQ (SPV-L3) and HLA-A, -B, and -C (W6/32) were ineffective (Table V). The inhibition by mAbs 135 and Q5/13 was specific since these mAbs did not affect the proliferative responses and the cytotoxic activity of $CD8^+$ HLA class I-specific T cell clones obtained from the patient (18). In addition, as will be shown in Table VIII, the response of the MHC-nonrestricted TT-specific T cell clone F3 was not blocked by mAbs Q5/13 and 135.

These data demonstrate that the T cell clones F11 and F13, derived from the first transplant (HLA-DR1,8) and the T cell clones F9, F15, and F17 derived from the

TABLE V

The Effect of Anti-HLA mAbs on the Proliferative Response of TT-specific HLA-restricted T Cell Clones

	Responder T cell clones				
mAb added*	F9	F11	F13	F15	F17
W6/32 (anti-HLA-A, B, C)	6‡	0	11	0	0
SPV-L3 (anti-HLA-DQ)	0	0	0	0	0
135 (anti-HLA-DR)	75§	85	86	90	97
Q5/13 (anti-HLA-DP, DR)	56	99	95	98	97
	26.4 ± 6.9‖	33.3 ± 2.2‖	53.8 ± 9.2‖	29.5 ± 2.4‖	248.3 ± 2.7‖

The host-derived EBV cell line SPS was used as APC.

* mAbs were used at a final dilution of 1:200 of ascites fluid.

‡ Percent inhibition of proliferation.

§ Significant inhibitions are underscored.

‖ Proliferative response in the absence of mAbs (cpm × 10^{-3}).

second transplant (HLA-DR3,9) both recognized TT presented in the context of HLA-DR4 or HLA-DR5 determinants of the recipient cells.

The T Cell Clones Restricted by the MHC of the Recipient Do not Recognize TT in the Context of Donor MHC. To exclude that the recognition of the Ag in the context of allo HLA determinants of the recipient by the TT-specific T cell clones was due to a crossreactivity between donor and recipient HLA determinants or to unknown HLA molecules shared between the two cell populations, we tested the ability of T cell clones of donor origin to recognize TT in the context of self HLA determinants. Since B cells and monocytes of donor origin were not available, we used an HTLV-1-transformed T cell clone (B21 p19) originated from the patient's PBL as APC. We recently showed that HTLV-1-transformed TT-specific T cell clones can process and present the Ag in an MHC-restricted fashion (Yssel, H., R. de Waal Malefyt, M. Duc Dodon, D. Blanchard, L. Gazzolo, J. E. De Vries, and H. Spits, submitted for publication). Therefore, we used this T cell clone, which is HLA identical with the TT-specific T cell clones F9, F15, and F17, to test the proliferative response to Ag in an autologous system.

As shown in Table VI, the T cell clones F9, F15, and F17 (HLA-DR3,9) recognized TT presented by SPS, but did not recognize the TT processed and presented by the T cell clone B21 p19, which shares the same HLA-DR3,9 determinants. In addition, they did not recognize TT presented by a second HTLV-1-transformed TT-specific T cell clone (827 p19), which was obtained from a normal donor and which shares the HLA-DR3 molecule. In contrast, B21 p19, as well as 827 p19, were able to present the TT to the nontransformed T cell clone 827, which is restricted by HLA-DR3. These responses were significant although lower than those obtained when the autologous EBV cell line HSY was used as APC. In the absence of TT or APC, no proliferation of the T cell clones was observed.

Analysis of MHC Nonrestricted TT-specific T Cell Clones. As already shown in Table III, the T cell clones F1, F3, and F14 proliferate to TT in the presence of both patient's and parents' fresh PBL. To determine the restriction element recognized by these clones, a study with a limited panel of EBV cell lines was performed. In Fig. 1, it is shown that clone F1 responded to TT when EBV cell lines sharing the HLA-

TABLE VI

Proliferative Response of HLA Class II-restricted T Cell Clones to TT Presented by HTLV-1-infected T Cell Clone B21p19

APC	HLA-DR phenotype	Responder T cell clones* F9 (DR 3,9)	F15 (DR 3,9)	F17 (DR 3,9)	827 (DR 3,7)
		cpm × 10^{-3}			
SPS	4.5	51.3‡	36.8	82.8	0.1
HSY	3.7	0.1	0.1	0.6	38.6
B21p19	3.9	0	0.1	0.1	13.2
827p19	3.7	0	0.6	0	23.8

* In all cases, the SD was <10% of total cpm and no [^{3}H]TdR incorporation was observed in the absence of TT.

‡ [^{3}H]TdR incorporation. Significant [^{3}H]TdR incorporation is underscored.

DR4 with the recipient were used. In addition, positive responses were observed with TT plus EBV cell lines bearing the HLA-DR1 antigen. These data indicate that the TT response of the clone F1 is restricted by HLA-DR4 and suggest a possible crossreactivity between the HLA-DR4 and HLA-DR1 molecules, which would explain why this clone reacted with both the cells of the father (HLA-DR4,7) and the mother (HLA-DR1,5).

Clones F3 and F14 were able to recognize TT presented by any EBV cell line tested, irrespective of their HLA phenotype (Fig. 1). The proliferative responses could also be induced by the EBV cell lines pulsed with TT for 18 h and subsequently washed (not shown), suggesting that these T cell clones recognized processed TT proteins on the surface of EBV cell lines with different HLA phenotypes. It could, however, not be excluded that the T cell clones recognize native TT just stuck to the membranes of the allogeneic EBV cell lines. To clarify this point, APC were pulsed with TT in the presence or absence of chloroquine, which inhibits intracellular Ag processing, and then fixed with glutaraldehyde to prevent any further processing. In Table VII, it is shown that the EBV cell line SPS pulsed with TT and

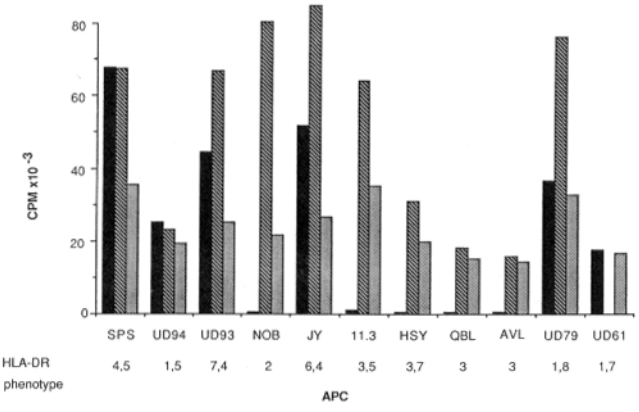

FIGURE 1. Proliferative response of HLA; nonrestricted T cell clones to TT presented by a panel of EBV cell lines. Solid bars, hatched bars, and grey bars represent [^{3}H]TdR incorporation (cpm × 10^{-3}) of the T cell clones F1, F3, and F14, respectively. T cell clone F3 was not tested with UD61 as APC. In all cases, the responses of T cells in the presence of TT or APC alone was <10^3 cpm and the SD was <10%.

TABLE VII

HLA Nonrestricted T Cell Clones Recognize Processed Antigen

Treatment of APC before fixation*	Responder T cell clones‡		
	F3	F14	F17§
		cpm × 10^{-3}	
-	0‖	0.1	0.5
TT 1:400	26.0	8.9	24.4
TT 1:1,000	20.7	10.2	25.9
Chloroquine + TT 1:400	2.7	2.6	14.5
Chloroquine + TT 1:1,000	0.2	0.5	5.7

* The host-derived EBV cell line SPS was used as APC.
‡ In all cases, the SD was <10% of the total cpm.
§ F17 recognizes TT in the context of HLA-DR5.
‖ [^{3}H]TdR incorporation.

then fixed with glutaraldehyde can present Ag to the T cell clones F3, F14, and F17. The addition of chloroquine during the Ag pulse resulted in strong reduction of proliferation of T cell clones F3 and F14. This inhibition was similar to that observed with the TT-specific T cell clone F17, which is HLA-DR5 restricted. These data indicate that the broadly reactive clones F3 and F14 recognize a processed TT protein on the surface of EBV cell lines.

To determine whether TT was recognized in the context of MHC determinants by these T cell clones, blocking studies with mAbs directed against class I and II HLA antigens were performed. Proliferation of clones F1 and F14 was significantly inhibited by mAbs 135 and Q5/13, which are specific for the class II HLA determinants. In contrast, the response of clone F3 was not inhibited by any of the mAbs tested (Table VIII).

Discussion

We studied a patient suffering from SCID who was successfully transplanted with two subsequent HLA-mismatched FLTT >10 yr ago. In this child, we observed a

TABLE VIII

The Effect of Anti-HLA mAbs on the Proliferative Response of TT-specific HLA Nonrestricted T Cell Clones

mAb added*	Responder T cell clones		
	F1	F3	F14
W6/32 (anti-HLA-A,B,C)	19‡	0	0
SPV-L3 (anti-HLA-DQ)	0	0	0
135 (anti-HLA-DR)	49§	0	47
Q5/13 (anti-HLA-DP, DR)	62	0	70
	79.3 ± 3.8‖	48.5 ± 0.9‖	12.5 ± 0.2‖

The host-derived EBV cell line SPS was used as APC.
* mAbs were used at a final dilution of 1:200 of ascites fluid.
‡ Percent inhibition of proliferation.
§ Significant inhibitions are underscored.
‖ Proliferative response in the absence of mAbs (cpm × 10^{-3}).

stable engraftment of T cells from the second transplant while the cells from the first transplant, initially present, progressively disappeared and eventually were not readily detectable in the peripheral blood. The B cells and monocytes remained of host origin. Despite this situation of split chimerism, the patient was able to mount normal in vivo antibody responses against thymus-dependent antigens such as TT (9, 10). In addition, TT-specific proliferative and antibody responses were observed in vitro (11). These data indicated that full immunological reconstitution could be achieved also when the transplanted cells are completely mismatched with the recipient and suggested a possible cooperation between donor-derived T cells and host cells across the allogeneic barrier.

It was therefore important to dissect this T cell–APC interaction at the clonal level, and for this reason, TT-specific T cell clones were generated from patient's PBL. Results shown in the present paper clearly demonstrate that Ag-specific T cell clones of donor origin were able to recognize TT in association with class II MHC determinants expressed by the recipient APC. Interestingly, HLA typing performed at the clonal level revealed that a proportion of these TT-specific T cell clones, which were able to proliferate to TT presented by the recipient cells, have the HLA phenotype of the first donor. This finding indicates that, in spite of the fact that T cells from the first transplant were not readily detectable in PBL by conventional HLA typing, these cells are still circulating and can become functional. The specificity of this Ag recognition in the context of host MHC Ags was clearly demonstrated by the observation that the T cell clones F1, F9, F11, F13, F15, and F17 proliferated in response to TT presented not only by the EBV cell line of the recipient, but also by the EBV cell lines of the parents and by allogeneic EBV cell lines sharing the relevant HLA antigens. The blocking experiments with mAbs specific for class I or class II HLA molecules confirmed that TT is recognized in the context of class II HLA. T cell clones F9, F15, and F17, which originated from the fetal cells of the second transplant, recognized Ag in the context of HLA-DR5; while HLA-DR4 was the restriction element of T cell clones F1, F11, and F13, the latter two were derived from the first donor.

The positive responses to TT obtained when fresh PBL of the patient and his parents were used as APC suggest that the Ag recognition observed in vitro reflects a system operational in vivo and is not related to the use of EBV cell lines as APC. Furthermore, the demonstration that these T cell clones are not able to proliferate to TT presented by self APC indicates that the Ag recognition in the context of the allo MHC observed in this patient is not due to a crossreactivity between the HLA determinants of the recipient and the transplanted T cells or to the presence of unidentified HLA molecules shared between the recipient and the donors. From these findings, it can be concluded that in this human chimera, there are two different populations of mature T cells (one, HLA-DR3,9, and the other, HLA-DR1,8) that have the capacity to recognize Ag in the context of HLA-DR5 and HLA-DR4 determinants of the recipient and do not recognize the Ag in the context of self.

These data demonstrate that recipient and donor cells bearing different HLA phenotypes cooperate across the allogeneic barrier and refute the hypothesis that in such cases interactions of T lymphocytes with cells of the HLA-mismatched hosts would be low or absent (19). Furthermore, our results can explain why transplantation of fully allogeneic stem cells results in the development of normal immune responses

even when a split chimerism is established. In addition, they indicate that HLA-restricted T cell recognition of T helper cells in human chimeras is adoptively generated under the influence of environmental MHC, rather than being dependent from the genotype of stem cells. Our data are in line with those obtained in murine lymphohaemopoietic chimeras (4, 5, 20, 21). In these chimeric animals, the thymus plays a central role in the education process, and the MHC phenotype of the thymus seems to determine the selection of the T cell repertoire (2, 6). In particular it has been recently shown that the MHC molecules expressed on the thymic epithelial cells specifically dictate the pattern of MHC restriction, while the MHC antigens expressed on the bone marrow-derived cells control the induction of tolerance (22). Results obtained in transplanted SCID mice also demonstrate that the bone marrow pro-T cells of donor origin could colonize and differentiate in the host thymus (23).

In this patient, it is not clear whether the acquisition of Ag recognition in the context of allo MHC determinants of the recipient is the result of an education process of transplanted fetal cells in the host thymus. In fact, in SCID children, data about repopulation of host thymus by the transplanted T cells are still fragmentary and controversial (24–27). Several reports indicate that transplanted T cells colonize the host thymus and suggest that immunoreconstitution has inductive or trophic effects on thymic development (24, 25). On the other hand, observations in an immunologically reconstituted SCID patient who died from an unrelated cause after transplantation showed little or no evidence of intrathymic differentiation of lymphocytes, while lymphocyte colonization and germinal center formation in peripheral lymphoid tissues was observed, suggesting an important role of extrathymic sites in the process of T cell education in self recognition (27). In addition, the observation that a SCID patient was successfully treated by transfusion of PBL from a histocompatible donor demonstrates that host thymic education is not a prerequisite to obtain a stable long-term immunological reconstitution (28).

We also isolated TT-specific T cell clones (F3 and F14) that are able to recognize processed Ag presented by a panel of allogeneic EBV cell lines irrespective of their HLA phenotype. The Ag-specific proliferative response of one of these clones could be blocked by the anti-class II mAbs indicating that it recognized Ag in the context of class II HLA molecules. Whether these MHC nonrestricted TT-specific T cell clones represent an alternative mechanism of Ag responsiveness developed during the differentiation of the fetal cells in an MHC-mismatched environment, or whether they result from an abnormal or incomplete thymic education, remains to be determined. It is interesting to note that both these MHC nonrestricted T cell clones have the HLA phenotype of the first transplant. The significance of this finding is presently unclear.

We recently demonstrated that no or incomplete clonal deletion of host-reactive T cells from the repertoire took place (18). In fact, a series of cytotoxic and proliferative T cell clones specific for the recipient cells has been isolated in vitro from patient's PBL. These clones were either $CD8^+$ and directed against class I MHC antigens, or $CD4^+$ and specific for class II MHC antigens (18). Therefore, this patient possesses precursors of host-reactive proliferative/cytotoxic effector cells and also precursors of effector cells capable of generating host-restricted antigen-specific responses. These findings underscore the fact that the mechanisms for deletion of self-reactive clones and the generation of MHC-restricted response are different. A recent hy-

pothesis put forward by Marrack et al. (22) predicts that self-reactive clones are eliminated in the host thymus when they come in contact with bone marrow-derived APC of recipient origin. Since precursors of self-reacting clones were not eliminated in this patient, our data imply that either the transplants matured in a host thymic environment that contained only host thymic epithelial cells and no bone marrow-derived cells or that antigen-specific MHC-restricted precursors are not educated in the thymus (29).

Finally, it has to be mentioned that in this patient the mechanism(s) responsible for the induction and maintenance of tolerance of the T cells derived from the two transplants towards each other and towards the non-T cells of the host remain unknown, but suggest the presence of important suppressor mechanisms.

Summary

Tetanus toxin (TT)-specific T cell clones of donor origin were obtained from a patient with severe combined immunodeficiency (SCID) successfully reconstituted by transplantation of allogeneic fetal liver and thymus cells from two different donors performed 10 yr ago. A series of these clones recognized TT in the context of "allo" class II HLA determinants expressed by recipient APC. The restriction element of two T cell clones with the HLA phenotype of the first donor (HLA-DR1,8) and one T cell clone with the HLA phenotype of the second transplant (HLA-DR3,9) was HLA-DR4 of the recipient, whereas other T cell clones derived from the second transplant recognized TT in the context of HLA-DR5 of the recipient's APC. These latter T cell clones were not able to proliferate in response to TT when autologous APC were used. These data demonstrate that recipient and donor cells having different HLA phenotypes could cooperate across the allogeneic barrier and that MHC restriction of antigen (Ag) recognition is independent from the MHC genotype of the T cells but is influenced by the environment in which the T cells mature.

We also isolated T cell clones that were able to recognize processed TT presented by all allogeneic EBV cell lines tested, indicating that the Ag specificity of these clones was not restricted by a particular class II MHC molecule. The Ag-specific proliferative response of one of these clones could be blocked by anti-class II MHC mAbs. These results demonstrate that in addition to Ag recognition in the context of specific class II MHC Ags, other types of Ag-specific responses may occur in this human chimera. It is not clear whether this "allo" plus Ag recognition is the result of education of transplanted fetal cells in the host thymus. Taking into consideration our previous findings indicating that alloreactive T cell clones specific for the recipient cells could be isolated in vitro from the PBL of the same patient, our data suggest that the mechanism for deletion of self-reactive clones and the generation of MHC-restricted responses are different.

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