0022-1767/90/1444-1288$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 144, 1288–1294, No. 4, February 15, 1990
Printed in U.S.A.

# ANALYSIS OF THE DONOR-SPECIFIC CYTOTOXIC T LYMPHOCYTE REPERTOIRE IN A PATIENT WITH A LONG TERM SURVIVING ALLOGRAFT

## Frequency, Specificity, and Phenotype of Donor-Reactive T Cell Receptor (TCR)-$\alpha\beta^+$ and TCR-$\gamma\delta^+$ Clones[1]

BART A. E. VANDEKERCKHOVE,[2]* GERT DATEMA,* FRITS KONING,* ELS GOULMY,* GUIDO G. PERSIJN,* JON J. VAN ROOD,* FRANS H. J. CLAAS,* AND JAN E. DE VRIES[3]†

*From the *Department of Immunohaematology and Blood Bank, University Hospital of Leiden, 2300 RC Leiden, The Netherlands, and †UNICET Laboratories for Immunological Research, 27 chemin des peupliers, BP 11, 69572 Dardilly, France*

**In the present study the transplant specific CTL repertoire of a patient (HLA:A1,3, B8,18, Cw5,7 DR3, DQw2, DPw3) with a long term surviving HLA mismatched kidney graft (HLA: A1,24 B8,27 Cw2,7, DR3,w13 DQw2,6 DPw1,3) has been investigated. This patient was unable to generate specific cytolytic activity against donor-derived PHA-blasts in the MLC in which donor spleen cells or B lymphoblastoid cell line were used as stimulator cells. In addition, the CTL precursor frequencies against donor alloantigens were very low (1/67,000). The patient had otherwise normal immune responses in vivo and in vitro and no signs of transplant rejection. Transplant specific CTL clones were generated in high frequencies (1/195) from T cell bulk cultures activated by PHA in the absence of any sensitization by donor Ag in vitro. The repertoire of 14 donor-reactive CTL clones (12 TCR-$\alpha\beta^+$ and 2 TCR-$\gamma\delta^+$) was analyzed. Two TCR-$\alpha\beta^+$ CD8$^+$ clones were specific for B27. Ten TCR-$\alpha\beta^+$ CTL clones directed against class II HLA Ag were isolated. Seven of these were CD4$^+$ and recognized DRw13 (3), DQw6 (3), and DPw1 (1), whereas three of these clones were CD4-CD8$^+$ recognizing DRw13 (1) and DQw6 (2). In addition, two donor-specific TCR-$\gamma\delta^+$ CTL clones were obtained recognizing HLA-A9(23,24) and DQw6. Our data indicate that the precursors of CTL clones specifically directed against donor class I or II HLA Ag are not deleted from the repertoire and that part of this reactivity resides in the TCR-$\gamma\delta^+$ fraction.**

Animal studies have shown that transplantation can induce tolerance to the alloantigens expressed on the transplant. Administration of cyclosporin (1), antithymocyte globulin (2), previous donor-specific transfusions (3) or infusion of donor-specific enhancing antibodies (4) in combination with allografting can result in some strain combinations in permanent acceptance of the grafts without further conditioning of the recipient. Although graft adaptation might occur in this process, tolerization of the recipient is also of importance, since these animals accept a subsequent graft of the same donor (5). This tolerance is donor-specific and in some experimental models tolerance can be transferred by donor-specific Ts cells (6).

Induction of transplantation tolerance in man is less well understood and the present knowledge is almost completely derived from in vitro studies and case reports. Such studies have shown that cessation of immunosuppressive therapy does not result in adverse effects in a proportion of the patients (7, 8). Furthermore, in about 70% of the patients no cytolytic activity against donor derived PHA-blasts can be generated in MLC 6 mo after transplantation (9, 10). This nonresponsiveness is specific for donor antigens. Other studies showed a decrease in the frequency of CTLp[4] against donor transplantation antigens after successful transplantation (11). Finally, some evidence for the involvement of a suppressor mechanism has been presented by Goulmy et al. (12) who showed that HLA-B locus Ag of the donor can down-regulate the lytic activity against any HLA-A Ag coexpressed on the stimulator cells.

To investigate the nature of this nonresponsiveness in man, one kidney transplant patient was studied in more detail. This patient was selected because she was in apparent good health nine years after transplantation of a poorly HLA-matched kidney graft and because she developed cell-mediated lympholysis nonresponsiveness in the years after transplantation. In the present study, we describe the isolation of a series of TCR-$\alpha\beta^+$ and TCR-$\gamma\delta^+$ CTL clones specific for donor class I or II Ag. In addition, limiting dilution experiments of PHA-activated PBMC indicated that precursors of these donor reactive CTL clones are present in high frequencies in this patient.

### MATERIALS AND METHODS

*Patient.* Patient JFV is a 38-yr-old woman (HLA:A1,3 B8,18, Cw5,7 DR3, DQw2, DPw3) who received a cadaveric renal allograft

Received for publication September 19, 1989.
Accepted for publication November 10, 1989.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was funded by the J. A. Cohen Institute for radiopathology and Radiation Protection. J. E. de Vries was appointed Boerhaave Professor at Leiden University.
[2] Correspondence and reprint requests should be addressed to: B. Vandekerckhove, Department of Immunohaematology and Blood Bank, Building I-E3-62g, University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands.
[3] Present address: DNAX Research Institute, Palo Alto, CA.
[4] Abbreviations used in this paper: CTLp, CTL precursor; B-LCL: B lymphoblastoid cell line; FMF, flow microfluorimetry.

of donor SP (HLA: A1,24, B8,27 Cw2,7, DR3,w13 DQw2,6 DPw1,3) in June 1979. Eight days after transplantation, a histologically proven rejection occurred which was treated successfully. There were no further complications except for multiple spinaliomas, which is the reason her medication was switched from azathioprin to oral cyclosporin (250 mg/day) in September 1987. At present, the patient is healthy, has a creatinin clearance of 96 ml/min, and no hypertension, proteinuria, or urine sediment abnormalities.

*Generation of B-LCL.* The EBV-transformed cell lines of the patient JFV, donor SP, and the panel members originated from infection of fresh PBMC or spleen cells (of the donor) with EBV obtained from the marmoset cell line B95-8. All cell lines were cultured in Yssel's medium supplemented with 1% pooled human $AB^+$ heat-inactivated serum designated culture medium.

*mAb.* The following mAb were used for blocking studies or FMF analysis: W6/32, B9.12.1 (13) and B1.23.2 (14), all directed against monomorphic HLA class I structures, B1.1G6 (15) anti-$\beta_2$-microglobulin, PdV5.2 (16) against a monomorphic epitope shared by DR,DP and most of the DQ alleles, IIB3 (17) anti-DQw1,8,9, B8.11.2 (18) anti-DR backbone, SPV-L3 (19) anti-DQ backbone, B7/21 (obtained from the Xth International Histocompatibility Workshop) anti-DP backbone, WT32 (anti-CD3), RIV6 (anti-CD4), FK18 (anti-CD8), WT31 (anti-TCR-$\alpha\beta$, gift of Dr. W. Tax, Radboudziekenhuis, Nijmegen), 11F2 (anti-$\gamma\delta$TCR, gift of Dr. J. Borst, Netherlands Cancer Institute, Amsterdam) (20), $\delta$TCS1 (T Cell Sciences, Cambridge, MA), BB3 (a gift of Dr. L. Moretta, National Cancer Institute, Genova) (21) and Ti$\gamma$A (gift of Dr. T. Hercend, Institut Gustave-Roussy, Villejuif) (22). All these mAb were used as diluted ascites. FITC or phycoerythrin-labeled Leu-2 (anti-CD8), Leu-3 (anti-CD4), Leu-4 (anti-CD3), Leu-16 (anti-CD20), anti-IL-2R (anti-CD25) and anti-TCR1 (WT31) were purchased from Becton Dickinson, Mountain View, CA.

*Mixed lymphocyte reaction.* Irradiated stimulator cells ($5 \cdot 10^4$ spleen cells or $10^4$ B-LCL in 50 $\mu$l culture medium) were incubated in microwells for 5 days with $5 \cdot 10^4$ responder cells in an equal volume in a humidified incubator of 5% $CO_2$. During the last 16 h 1 $\mu$Ci of tritiated thymidine was added. Subsequently the cultures were harvested onto fiber glass filters and tritiated thymidine incorporation was determined by liquid scintillation spectroscopy.

*Cytotoxic assay.* Appropriate numbers of effector cells (cloned T cells or T cell bulk cultures) were mixed with $2 \cdot 10^3$ $^{51}$Cr-labeled target cells (PHA-blasts or B-LCL cell lines) in 0.2 ml of culture medium in U-shaped microtiter wells. The plates were centrifuged (600 × *g*, 1 min) and incubated for 4 h at 37°C in a humidified atmosphere of 5% $CO_2$. The supernatants were harvested with a Skatron harvesting system and counted in a gamma counter. Percentage of lysis was calculated according to the formula:

$$\text{Percent lysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \cdot 100.$$

Maximal release was determined from a saponine lysate of the target cells. Spontaneous release was determined from at least eight cultures containing target cells only.

Blocking of cytotoxic activity with mAb was carried out as follows: a hundredfold dilution of the mAb containing ascites was preincubated with the target cells or effector cells in 0.1 ml at 37°C. After 30 min effector cells or target cells were added without prior washing.

*Limiting dilution analysis.* The analysis of CTLp frequencies in a limiting dilution culture was performed as described previously (23). Briefly, graded numbers of PBMC from the recipient were cultured in the presence of $5 \cdot 10^4$ irradiated spleen cells or PBMC (3000 rad) as stimulator cells in a total volume of 0.2 ml of culture medium. Twenty-four wells were analyzed for each responder cell concentration. rIL-2 was added as a growth factor. After 7 days of culture each microtiter well was tested for cytolytic activity by replacing 0.1 ml culture medium by 5000 $^{51}$Cr-labeled PHA target cells in the same volume. After mixing and centrifugation the cultures were treated as described above. Cultures were considered to be positive when the $^{51}$Cr release exceeded the spontaneous release (mean of 24 cultures) plus 3 SD. Frequencies of CTLp, 95% confidence interval and *p* value were calculated as described (23).

*Generation of donor-specific CTL clones from PHA-blasts.* PBMC ($10^6$/ml) were stimulated with PHA (0.1 $\mu$g/ml) for 4 days. The activated cells were layered on a Ficoll gradient and centrifuged (15 min, 600 × *g*). Living cells were collected from the interface, washed three times, and cloned by limiting dilution at 1 cell in every three wells in 96-well U-shaped microtiter plates in volumes of 0.1 ml in the presence a feeder cell mixture consisting of $10^6$ irradiated PBMC (4000 rad) and $10^5$ irradiated B-LCL (5000 rad) and 0.1 $\mu$g PHA/ml. HLA-typed feeder cells were used in order to ensure that the feeder cell mixture did not share any HLA Ag with the donor. In order to enhance specific cytotoxic activity and to reduce simultaneously nonspecific cytotoxicity (24, 25), IL-2 (20 IU) and IL-4 (100 U) were added as growth factors. After 14 days of culture in a humidified incubator of 5% $CO_2$ at 37°C, growing cultures were divided into three equal parts in U-shaped microwells. Two of the three wells were assayed for cytotoxic activity against the donor B-LCL. Cultures that scored positive (mean of the duplo determination above the spontaneous $^{51}$Cr release plus 3 SD) in the cell-mediated lympholysis assay were transferred to a volume of 1 ml in a 24-well Costar plate and restimulated with a feeder cell mixture in the presence of IL-2 and IL-4.

*Generation of donor-specific CTL clones from PBMC.* PBMC of the patient were seeded by limiting dilution at 500 cells/well in 96-well U-shaped microtiter plates. To each well $10^4$ irradiated donor B-LCL (5000 rad) were added in a final volume of 0.2 ml. One hundred U of IL-4 was added as a growth factor. After 7 days, 0.1 ml of medium was replaced by fresh medium, containing in addition to IL-4, 20 IU of IL-2. After an additional week at 37°C, growing cultures were tested for cytotoxic activity against the donor PHA-blasts as described above and subcloned at one cell in every three wells. Specific CTL clones were transferred to a 24-well Costar plate in volumes of 1 ml and restimulated with the feeder cell mixture in the presence of IL-2 and IL-4.

*Proliferation assay.* Between 9 and 14 days after the last restimulation, clones were tested for proliferation as follows: $2 \cdot 10^4$ clone cells were incubated with an equal number of the relevant irradiated B-LCL (5000 rad) in U-shaped microtiter wells. The cultures were incubated for 3 days at 37°C in a humidified atmosphere of 5% $CO_2$. During the last 16 hours, one $\mu$Ci of tritiated thymidine was added. Subsequently the cultures were harvested onto fiber glass filters and tritiated thymidine incorporation was determined by liquid scintillation spectroscopy.

*FMF analysis.* One hundred thousand cells were labeled with mAb and FITC-labeled goat anti-mouse (Becton Dickinson) according to the standard procedure described previously (26). For double labeling, cells were incubated with the appropriate mAb, one FITC-labeled and the other phycoerythrin-labeled, in PBS with 0.1% BSA and 0.1% sodium azide for 30 min. The samples were analyzed on a FACScan (Becton Dickinson).

## RESULTS

*Immunologic status of the patient.* The patient had normal lymphocyte (1800/mm$^3$) and monocyte (500/mm$^3$) counts. FMF analysis of the PBMC showed that 77% were $CD3^+$ T cells, 74% TCR-$\alpha\beta^+$ and 2.4% TCR-$\gamma\delta^+$ T cells. The patient had a normal $CD4^+/CD8^+$ ratio of 2.0 (44% vs 22%). There were no signs of T lymphocyte activation in vivo: 1.3% $CD3^+DR^+$ and 0.5% $CD3^+CD25^+$ circulating cells were observed. In addition, the spontaneous thymidine incorporation by PBMC of the patient was comparable to that of PBMC from healthy control donors. Furthermore, the proliferative responses to PHA and anti-CD3 mAb were in the normal range (not shown).

*Proliferative and cytotoxic activity against donor alloantigens.* To determine the proliferative and cytotoxic capacity of PBMC of the patient, MLR were carried out in which irradiated spleen mononuclear cells or B-LCL of donor SP were used as stimulator cells. In Table I it is shown that the PBMC of the patient proliferated in response to both the B-LCL and the spleen MNC of donor SP. The proliferative responses were in the normal range, as compared with 3rd party stimulator cells tested in

TABLE I

*MLR responses in the presence or absence of IL-2 and/or IL-4*

| Lymphokine Added[a] | Stimulator Cells: [$^3$H]TdR Incorporation (cpm.$10^{-3}$) with | | |
|---|---|---|---|
| | SP-B-LCL cells | SP-Spleen cells | 3rd party B-LCL cells |
| | 45[b] | 12 | 40–121 |
| IL-4 | 91 | 27 | 85–119 |
| IL-2 | 91 | 41 | 70–128 |
| IL-2 + 4 | 93 | ND | 86–106 |

[a] IL-2 (20 IU/ml) and/or IL-4 (100 U/ml) were added at the onset of the culture.

[b] Spontaneous thymidine incorporation of irradiated stimulator cells or responder cells were always lower than 1000 cpm.

parallel and were enhanced by IL-2, IL-4, or combinations of IL-2 and IL-4.

The cytolytic activity against PHA-blasts of SP before transplantation varied between 25 and 40%, but decreased gradually and was negative 2 yr after transplantation (Table II). This donor-specific nonresponsiveness was still present at the time of this study (>9 yr after transplantation). In contrast, cytolytic activity induced by third party stimulator cells resulted in low levels of specific CTL-activity before and shortly after transplantation and higher levels thereafter, probably reflecting the improving general condition of the patient after transplantation. In Table III, it is shown that when B-LCL are used as stimulator cells the cytotoxic response remains negative, however, a low degree of donor-specific CTL activity against PHA-blasts of SP was measured after secondary MLC stimulation. The specific CTL activity was not enhanced when MLC were carried out in the presence of IL-2, IL-4 or a combination of IL-2 and IL-4 (not shown).

*Frequency of donor-specific CTLp.* To determine the frequency of donor-specific CTLp, limiting dilution analyses were carried out. Various numbers of PBMC of the patient were cultured for 7 days as indicated in materials and methods and tested against the specific target cell. The CTLp frequencies as measured against donor PHA-blasts were low: 1/67000, while CTLp frequencies against a 3th party control were within the normal range: 1/5500 (Fig. 1).

To exclude the possibility that the frequency determinations were influenced by suppressive mechanisms, CTLp frequencies against donor antigens were also determined at the clonal level. PBMC of the patient, activated for 4 days by PHA and cloned by limiting dilution at one cell in every three wells, resulted in 1167 growing cloned T cell lines. Six of these 1167 T cell lines were specifically cytotoxic for the SP PHA-blasts, accounting

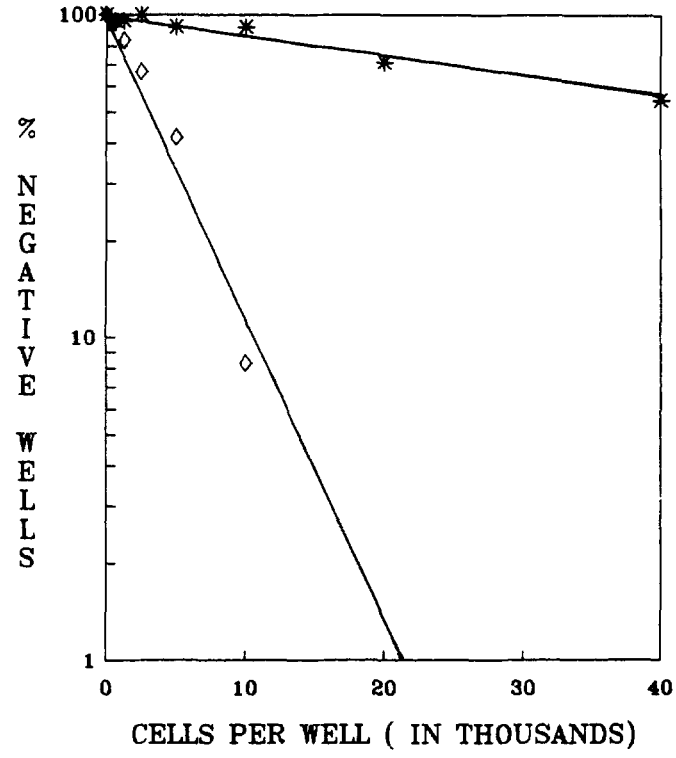


*Figure 1.* CTLp frequencies in the blood of patient JFV against the donor SP (*) and against a third party stimulator cell (◇). The *p* value for single hit kinetics was always >0.05.

for a precursor frequency of 1/195. The CTLp frequency determinations at the clonal level were carried out with typed feeder cells not sharing any HLA Ag with the donor. Therefore, these results demonstrate that donor-reactive CTLp are present in the circulation of this patient at relatively high frequencies.

*Isolation of CTL clones specifically recognizing kidney donor MHC Ag.* In a second series of experiments donor-specific CTL clones were generated by seeding unstimulated PBMC of the patient at 500 cells per well in the presence of the SP B-LCL and IL-4. Eight proliferating cultures that contained lytic activity specifically directed against SP were obtained and further subcloned at one cell in every three wells. The phenotype, proliferative and lytic properties of these eight CTL clones together with the six clones established after activation by PHA as described above are shown in Table IV. None of these clones lysed autologous patient derived B-LCL cells. Seven CTL clones were TCR-$\alpha\beta^+$ $CD4^+$. In addition to their specific cytotoxic activities these clones also proliferated specifically in response to donor antigens. The cytotoxic activity against SP-BLCL of the $CD4^+$ clone, X13, was weak and varied upon repetitive testing, but clone X13 was consistently found to be donor-specific as determined in proliferation assays (Table V). In contrast to the $CD4^+$ CTL clones, the TCR-$\alpha\beta^+$ $CD8^+CD4^-$ CTL clones generally showed no or only weak proliferative responses to donor Ag (Table IV). Two donor-specific TCR-$\gamma\delta^+$ CTL clones (21 and 40) were obtained. Phenotypic analysis demonstrated that both clones were $CD2^+$,$CD3^+$, $CD4^-$. CD8 was expressed on 30 to 60% of the cloned 40 cells and was weak or absent on clone 21. This CD8 expression, as the CD8 expression on the TCR-$\alpha\beta^+$ $CD4^+$ clones, was induced by IL-4 present in the cultures (27) and was lost when the clones were cultured

TABLE II

*Loss of donor-specific cytotoxic activity after transplantation*

| Time Point Relative to Transplantation | Induction Culture (Stimulator-Responder)[a] | | |
|---|---|---|---|
| | SP-JFV | 3rd party-JFV | SP-3rd party |
| −256 days | 40[b] | 19 | ND |
| 0 days | 25 | 14 | ND |
| 14 days | 17 | 6 | ND |
| 1 yr | 11 | 48 | 30 |
| 2 yr | −1 | 80 | 52 |
| 9 yr | −1 | 30 | 18 |

[a] The induction cultures were set up with spleen cells of the donor or PBMC of 3rd party controls as stimulator cells. After 7 days they were assayed for cytotoxic activity against PHA-blasts of the stimulator cell used in the induction culture.

[b] Percent lysis at a 50 to 1 E:T ratio.

TABLE III

*Donor(SP)-specific lysis by primary and secondary MLR cultures*

| Induction Culture | | | Percent Lysis of Target Cells | |
|---|---|---|---|---|
| SP cells used | Responder | 1st/2nd stimulation | SP PHA blasts | Autologous PHA blasts[a] |
| BLCL | JFV | 1 | 1 | −2 |
| BLCL | 3rd party[b] | 1 | 18 | 0 |
| Spleen | JFV | 1 | −1 | −2 |
| Spleen | 3rd party | 1 | 18 | 0 |
| BLCL | JFV | 2 | 12 | 0 |
| Spleen | JFV | 2 | 10 | −1 |
| Spleen | 3rd party | 2 | 65 | 2 |

[a] Autologous to the responder of the induction culture.

[b] 3d party responder: PBMC of a healthy individual sharing no HLA Ag with the donor cells.

TABLE IV

*Phenotype, proliferative and cytotoxic responses of donor-specific clones*

| Clone[a] | Phenotype | | | Proliferation[b] (cpm·10⁻³) | | Percent lysis (10:1) | |
|---|---|---|---|---|---|---|---|
| | TCR | CD4 | CD8 | SP | JVF | SP | JVF |
| 1. P25 | αβ | 1 | 100 | 2.1 | 1.5 | 88 | 2 |
| 2. X7 | αβ | 0 | 95 | 11.1 | 0.9 | 37 | 1 |
| 3. X212 | αβ | 2 | 100 | 7.5 | 0.7 | 38 | 0 |
| 4. X211 | αβ | 97 | 0 | 12.5 | 0.8 | 27 | 4 |
| 5. X13 | αβ | 100 | 50 | 12.2 | 0.9 | 15 | −1 |
| 6. T7 | αβ | 100 | 39 | 68.1 | 1.2 | 28 | −2 |
| 7. 33 | αβ | 0 | 95 | 2.0 | 1.3 | 65 | 1 |
| 8. 15 | αβ | 0 | 100 | 4.9 | 1.0 | 80 | 3 |
| 9. 31 | αβ | 100 | 1 | 60.1 | 0.6 | 59 | −2 |
| 10. 36 | αβ | 100 | 24 | 50.5 | 0.8 | 63 | 0 |
| 11. 26 | αβ | 100 | 38 | 35.5 | 0.8 | 65 | −1 |
| 12. 3 | αβ | 100 | 27 | 86.4 | 1.2 | 25 | 0 |
| 13. 21 | γδ | 0 | 11 | 1.5 | 1.5 | 26 | 0 |
| 14. 40 | γδ | 0 | 58 | 3.5 | 1.7 | 41 | 0 |

[a] Clones 1 to 6 are the PHA derived clones, clones 7 to 14 were obtained by allostimulation of PBMC.

[b] Spontaneous thymidine incorporation of the clones was always lower than 1000 cpm and the spontaneous incorporation of the stimulator cells was always lower than 3000 cpm.

TABLE V

*Specificity of proliferation of X13*

| Stimulator | | | Proliferation ([³H]TdR cpm·10³) of X13 in Presence of (mAb): | | | |
|---|---|---|---|---|---|---|
| Code | Shared HLA Ag | Background proliferation | None | SPV-L3 (anti-DQ) | B7/21 (anti-DP) | B8.11.2 (anti-DR) |
| SP | | 2, 4 | 12, 2 | 2, 8 | 17, 6 | 33, 2 |
| JFV | None | 0, 5 | 0, 7 | 1, 1 | 0, 7 | 0, 7 |
| MAST | DQw6 | 0, 9 | 7, 3 | 1, 1 | 7, 2 | 9, 7 |
| MVL | B27, Cw2 | 0, 6 | 1, 5 | | | |
| DKB | A24 | 2, 9 | 3, 5 | | | |

in IL-2 in the absence of IL-4 (not shown). Furthermore, both clones expressed a TCR-γδ that is δTCS-1⁺, TiyA⁻ and BB3⁻, indicating the use of the $V\delta_1J\delta_1$ in these receptors (28). (F. Koning, unpublished results). SDS-PAGE analysis of the receptor complex of clone 40 after immunoprecipitation showed the lack of a disulfide bond between the γ and δ chains (approximate m.w.: γ: 44,000, δ:41,000), indicating the use of a Cγ2 gene segment in this receptor (not shown).

*Specificity of the donor-specific TCR-αβ⁺ CTL clones.* In order to determine the specificity of the TCR-αβ⁺ CTL clones, blocking studies with mAb against class I and II MHC Ag (Tables V and VI) and limited panel studies (Tables V and VII) were carried out. All seven CD4⁺ CTL clones recognized class II HLA Ag. One CTL clone was specific for DPw1, three CTL clones recognized DRw13, and three CTL clones reacted specifically with DQw6. Interestingly, three CD8⁺ CTL clones had the "wrong" phenotype, because they were specific for HLA class II Ag: one CD8⁺ CTL clone (15) recognized DRw13 and the other two CD8⁺ CTL clones (X7,33) recognized DQw6. The DQw6-specific CTL clones did not lyse every DQw6⁺ target cell in the panels and are probably directed to a subtype of DQw6 as yet not detected by serology. This notion is supported by the observation that only DQw6⁺ target cells were lysed and that this reactivity was completely blocked by the anti-DQw1(w6) mAb IIB3. The remaining two CD8⁺ CTL clones recognized B27.

*Specificity of the donor-specific TCR-γδ⁺ clones.* Panel studies carried out with clone 40 indicated that 12 out of 13 HLA-A24⁺ B-LCL were lysed (Table VIII). One A23⁺ (which constitutes together with the more frequent A24 allele the HLA-A9 specificity) B-LCL was also recognized. In contrast, none of the 12 A9⁻ B-LCL cells were lysed, strongly suggesting that the clone recognized the HLA-A9 specificity or the product of a closely linked gene. However, the cytotoxic activity could not be blocked by three different anti-HLA class I specific mAb: W6/32, B9.12.1 and B1.23.2 (Fig. 2*A*). Similarly, an anti-$\beta_2$-microglobulin mAb, B1.1G6, which should inhibit all class I and class I-like specific lysis, was also unable to inhibit cytotoxicity. Instead, an enhanced lysis in the presence of these mAb was observed. The enhanced lysis was not due to antibody-dependent cellular cytotoxicity, since A9⁻ targets, coated with anti-class I MHC mAb, were never lysed (not shown). The lack of inhibition was observed using various concentrations of mAb, various numbers of cloned effector cells and various types of A9⁺ target cells (B-LCL or PHA-blasts of three different panel members). Only when mAb B1.1G6 and W6/32 were added together, a low degree of inhibition was measured (35%, $p < 0.01$). Furthermore, anti-class II, -DR, -DQ, -DP mAb did not block clone 40, excluding the possibility that A9 peptides were recognized in the context of an HLA class II molecule. Complete blocking was obtained with the anti-TCR-γδ⁺ and the anti-CD3 mAb. The IL-4-induced CD8 expression did not contribute to the specificity or affinity of this clone, since the anti-CD8 mAb, FK18, was unable to prevent lysis by this clone. Furthermore, the CD8⁺ and CD8⁻ fractions, separated by FMF, lysed the donor B-LCL cells equally well (data not shown).

Blocking studies showed that the reactivity of clone 21 was strongly inhibited by the anti-class II HLA backbone and HLA-DQ mAb, whereas mAb against HLA-DR and HLA-DP were ineffective. The specific reactivity of clone 21 was also inhibited by mAb directed against the TCR-γδ⁺ (50%) and CD3 complex (Fig. 2*B*). Limited panel stud-

TABLE VI

*Inhibition of donor-specific cytotoxic activity of the TCR-αβ⁺ clones by anti-HLA mAb[a]*

| mAb | | Clones | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | Specificity | P25 | X7 | X212 | X211 | T7 | 33 | 15 | 31 | 36 | 26 | 3 |
| B9.12.1 | Class I | 100 | ND | 100 | 0 | ND | 0 | 0 | 0 | ND | 0 | ND |
| B1.23.2 | Class I | ND | ND | ND | ND | ND | 0 | 0 | 0 | ND | 0 | ND |
| B1.1G6 | $\beta_2$-Micro-globulin | 75 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pdv5.2 | Class II | 0 | 100 | 0 | 70 | 70 | 100 | 100 | 100 | 100 | 20 | 100 |
| B8.12.1 | DR | 0 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| SPV-L3 | DQ | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 |
| IIB3 | DQw1, 8, 9 | ND | 100 | 0 | 0 | ND | 100 | 0 | 100 | ND | 75 | ND |
| B7/21 | DP | 0 | 0 | 0 | 0 | 70 | 0 | 0 | 0 | 0 | 0 | 0 |
| Specificity: | | ABC | DQ | ABC | DR | DP | DQ | DR | DQ | DR | DQ | DR |

[a] Results are expressed as percent inhibition. A percentage of inhibition between <0 and 10% and between 90 and 100% was depicted as 0 and 100%, respectively, for ease of survey.

TABLE VII

*Specificity of the donor-reactive TCR-αβ⁺ CTL clones as determined by panel studies*[a]

| Targets | | Clones | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | Shared Ag | P25 | X7 | X212 | X211 | T7 | 33 | 15 | 31 | 36 | 26 | 3 |
| Donor | A24, B27, Cw2 DRw13, DQw6, DPw1 | 88 | 37 | 38 | 27 | 28 | 65 | 80 | 59 | 63 | 65 | 15 |
| Mast | DQw6 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 39 | −2 | 81 | −1 |
| ZUUR | A24, DQw6 | 0 | 18 | ND | ND | ND | 0 | 0 | 0 | 0 | 61 | 0 |
| BROE | DRw13, DQw6 | 0 | 62 | 2 | 80 | 4 | 52 | 83 | 65 | 68 | 87 | 24 |
| MVL | B27, Cw2 | 81 | ND | 24 | −1 | ND | 0 | 0 | 0 | 0 | 0 | 0 |
| PESA | B27, DRw13, DQw6 | 59 | 42 | 22 | 38 | ND | 0 | 19 | 13 | 46 | 63 | 25 |
| HHK | DRw13, DQw6 | 0 | 36 | ND | ND | 2 | 38 | ND | 49 | ND | 32 | ND |
| DKB | A24 | 0 | 2 | 0 | −5 | 4 | 2 | ND | 0 | ND | 0 | ND |
| PLIC | DPw1 | ND | ND | 3 | −1 | 25 | ND | ND | ND | ND | ND | ND |
| HAAN | DQw6 | ND | ND | 4 | 2 | −1 | ND | ND | ND | ND | ND | ND |
| BAKK | DQw6 | ND | ND | 0 | 3 | 2 | ND | ND | ND | ND | ND | ND |
| ABEL | DPw1, DQw6 | ND | ND | 0 | 0 | 24 | ND | ND | ND | ND | ND | ND |
| Specificity: | | B27 | DQw6? | B27 | DRw13 | DPw1 | DQw6? DRw13? | DRw13 | DQw6? | DRw13 | DQw6 | DRw13 |

[a] Results are expressed as percent lysis at a 10:1 E:T ratio.

TABLE VIII

*Specificity of the TCR-γδ⁺ clones 40 and 21 as determined by panel studies*[a]

| HLA Typings of Targets | | | | | | | Percent Lysis by Clone | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 40.1 | | 21.1 | |
| A | B | Cw | Dw | DR | DQw | DPw | B-LCL | PHA | B-LCL | PHA |
| 1, 24 | 8, 27 | 2, 7 | | 3, 13 | 2, 6 | 1, 3 | [42] | 18 | [26] | 10 |
| 1 | 8 | 7 | 3, 24 | 3 | 2 | 4 | 0 | 0 | 1 | |
| 24 | 51 | | 25 | 11 | 7 | 4 | [−5] | | | |
| 2, 24 | 27, 62 | 3 | | 4, 6 | | | [27] | | | |
| 2, 24 | 27, 62 | 3 | | 4, 6 | | | [39] | | | |
| 24 | 51, 63 | | 5, 25 | 11 | 7 | 2 | [18] | | | |
| 28, 30 | 18, 60 | 3, 5 | 18 | 3, 13 | 6, 2 | | 0 | | [15] | |
| 2 | 62 | 3 | 4 | 4 | 3 | 2 | 4 | | | |
| 2, 24 | 35 | 3, 4 | | 2, 4 | 2, 3 | | [45] | | | |
| 24, 28 | 35, 42 | 3 | | 3, 4 | 3 | | [34] | | | |
| 24 | 60 | 10 | 23 | 9 | 9 | 4 | [50] | 12 | −1 | |
| 3, 24 | 7, 55 | 3, 7 | 19 | 13, 8 | 6 | 2, 4 | [57] | | | |
| 3 | 7 | 7 | 18, 24 | 13 | 6 | 4 | 0 | | | |
| 1 | 35 | 4 | 5, 25 | 11 | 7 | | 0 | | | |
| 24 | 51 | | 1 | 1 | 5 | | [15] | | | |
| 24 | 38, 35 | | 18 | 2, 13 | 6 | | [16] | | | |
| 2 | 51 | | 8, 3 | 8 | 7 | 1, 4 | 2 | | | |
| 1, 3 | 37 | 6 | | 2, 10 | 5, 6 | 3, 4 | −1 | 0 | [16] | |
| 32 | 27 | 2 | 1 | 1 | 5 | 2 | 2 | | | |
| 3, 11 | 12, 16 | | | 2 | 2 | | −1 | | | |
| 1, 26 | 55, 27 | 1, 3 | 18 | 1, 13 | 6 | 2, 4 | 0 | | [48] | |
| 24 | 7 | 7 | 2 | 2 | 6 | 2 | [59] | 15 | | |
| 1, 2 | 8, 60 | 3, 7 | | 3, 4 | 2, 3 | 2, 4 | −4 | | | |
| 23 | 7 | 7 | | 2 | 6 | 2 | [49] | 18 | | |
| 1, 24 | 7, 8 | 7 | | 2, 3 | 6, 2 | 1, 4 | [18] | | | |
| 1, 3 | 8, 18 | 5, 7 | | 3 | 2 | | 0 | −1 | 0 | −1 |

[a] The lysis of A9 positive B-LCL is boxed for clone 40 and of DQw6 positive B-LCL for clone 21.

ies carried out with B-LCL targets indicated that clone 21 recognized DQw6 (Table VIII).

## DISCUSSION

In the present study we demonstrated that CTL clones specific for donor class I or II HLA Ag can be isolated at relatively high frequencies from a patient who had been successfully transplanted with a kidney more than 9 yr ago. This patient was selected because she was in good health with an excellent graft function and no signs of graft rejection, in spite of a poor HLA-match. Furthermore, it was established that her PBMC taken before transplantation, had normal proliferative and cytotoxic reactivities against donor transplantation antigens, whereas donor-specific cytotoxicity disappeared gradually after transplantation. No donor-specific cytotoxicity could be measured after 2 yr. The proliferative capacity toward donor derived stimulator cells remained intact. Similar data have been obtained in experimental animals with long term surviving organ allografts. Also in these models the donor-specific proliferative responses remained positive, whereas donor-specific cytotoxic activity was absent (6, 29).

In addition, the CTLp frequencies in our patient were specifically low against donor antigens, whereas the CTLp frequencies against 3rd party stimulator cells were in the normal range (which is between 1/2000 and 1/15000, depending on the individual tested) (23), suggesting that only the donor-specific CTLp frequencies decreased in this patient after transplantation. This is in agreement with the results of Herzog et al. (11), who reported decreased donor-specific CTLp frequencies after

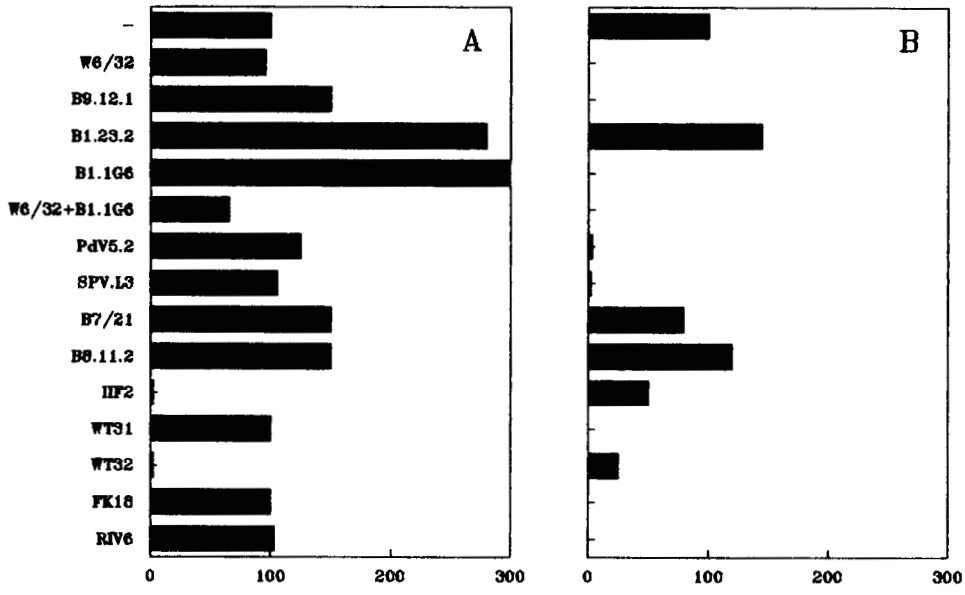


*Figure 2.* Effect of various mAb on the cytolytic activity of clone 40 (A) and clone 21 (B). These are the results of two representative experiments in which the 100% value for clone 40 was 32 and 48%, for clone 21, 41% and 26%.

transplantation in six kidney patients in whom rejection did not occur. It has to be noted, however, that a low degree of cytotoxic activity could be generated in secondary MLC.

In contrast, donor-specific CTL clones were obtained at high frequencies after activation by PHA and limiting dilution in the presence of HLA-typed feeder cells not sharing any HLA antigens with the kidney donor, indicating that such CTL clones could be obtained without sensitization by donor alloantigen in vitro. These results demonstrate unambiguously that CTLp are not deleted from the T cell repertoire. In this respect, our results are analogous to those obtained in experimental neonatal tolerance models, in which it has been shown that CTLp frequencies against tolerated alloantigens are dramatically reduced when measured by classical limiting dilution analysis. However, in the latter system (as in our patient), the donor-specific CTLp frequencies measured after culturing of limiting numbers of mitogen activated responder cells were high (1/100), comparable to or even higher than the frequencies measured in naive non-tolerant animals (30, 31). The finding that donor-specific CTLp frequencies as measured by classical limiting dilution assays were very low, whereas donor-specific CTLp frequencies as measured after activation by PHA as well as CTLp frequencies against 3rd party antigens were in the normal range, strongly suggests the presence of suppressor activities only affecting the generation of donor-specific cytolytic activity. An alternative explanation could be, that the transplant specific CTLp have special growth requirements which were provided by PHA stimulation in vitro, but which are absent upon activation by donor alloantigens in vitro or in the microenvironment in vivo.

The specificity, phenotype and functional properties of 14 donor-specific CTL clones were analysed. In addition to the TCR-$\alpha\beta^+$ $CD4^+$ CTL clones recognizing the donor class II HLA Ag DQw6, DRw13, and DPw1, one TCR-$\gamma\delta^+$ clone and a relatively high number of TCR-$\alpha\beta^+$ $CD8^+$ CTL clones were isolated, which also recognized HLA class II Ag of the donor. This class II specificity of the CTL clones lacking CD4 indicates that CD4 is not absolutely required for effective interaction with class II HLA Ag and suggests the presence of high affinity TCR (13, 32), which is thought to be a specific property of CTL clones activated in vivo (33, 34). Although, some of the $CD4^+$ donor-reactive CTL clones expressed CD8 which was—as we have shown previously (27)—induced by IL-4, no double positive clones were found that were specific for class I HLA Ag. IL-4 induced CD8 was also not involved in the specific recognition of A9 by the A9 specific TCR-$\gamma\delta^+$ clone. In addition, two TCR-$\alpha\beta^+$ $CD8^+$ CTL clones recognizing the donor class I HLA Ag B27 were obtained.

Donor-specific clones were found against all mismatched HLA Ag, except HLA-C: one CTL clone was directed against HLA-A9, 2 clones were specific for B27, 4 clones were specific for DRw13, 6 reacted with DQw6, and 1 CTL clone was specific for DPw1. This pattern of reactivity is very similar to the donor-reactive repertoire of graft infiltrating cells at the time of an acute irreversible rejection, described by Bonneville et al. (34), indicating that the donor-specific CTL repertoire in our "tolerant" patient is not changed in specificity. Changes in the specificity of the repertoire after neonatal tolerization (shift from H-2K to H-2D) have been described in laboratory animals by Wood et al. (35). Furthermore, these authors also found that a relatively large proportion of the graft infiltrating donor class II reactive cells was $CD8^+$ (2/14, compared with 3/12 in our series). However, in contrast to their findings, we found that 2/14 donor reactive clones were TCR-$\gamma\delta^+$. These clones were obtained without prior depletion for TCR-$\alpha\beta^+$ cells, indicating that part of the donor reactivity resides in the TCR-$\gamma\delta^+$ fraction.

Although alloreactive TCR-$\gamma\delta^+$ cells have been described in mice (36) and man (37), naive TCR-$\gamma\delta^+$ cells seems unable to mount an alloreactive response in vivo, as was demonstrated in nude mice. However, prolonged contact with alloantigens in vivo may upregulate the frequency of alloreactive TCR-$\gamma\delta^+$ cells (36). This may be the case in our patient. This notion is also supported by our finding that T cell cultures established from cardiac allograft biopsies taken 2 to 3 yr after transplantation contained in some patients a relatively high percentage of TCR-$\gamma\delta^+$ cells. TCR-$\gamma\delta^+$ cells are never found in the early posttransplantation period (N. Jutte et al. manu-

script in preparation).

The present data indicate that both TCR-$\alpha\beta^+$ and TCR-$\gamma\delta^+$ CTL clones specific for class I or class II HLA Ag expressed on the kidney can be isolated directly from the peripheral blood of the patient. Despite the relative high frequencies of CTL clones specific for HLA Ag expressed on the transplanted kidney, no signs of graft rejection were observed in this patient, indicating that these transplant-specific CTL clones seem not to be operational in vivo. This may be due to the maintenance immunosuppressive therapy, initially imuran and since 1987 cyclosporin A, which may prevent the maturation and expansion of any CTLp to a functionally mature CTL. On the other hand, specific suppressor mechanisms not affecting normal immune responsiveness, may account for the absence of transplant specific activity in vivo, which is probably also the reason why some transplanted patients continue to do well without rejection of the transplant after immunosuppressive therapy was stopped (7, 8). The latter possibility is supported by the finding that the patient described here had strongly reduced donor-specific reactivity whereas normal proliferative and cytotoxic activity against 3rd party alloantigens was found. This is difficult to explain by the action of cyclosporin alone.

A final answer to the question as to whether specific suppressor mechanisms are operational in patients with long term surviving HLA-mismatched transplants has to come from similar studies as described here in patients who continue to do well after immunosuppressive therapy is discontinued.

## REFERENCES


1. **Nagao, T., D. J. G. White, and R. Y. Calne.** 1982. Kinetics of unresponsiveness induced by a short course of cyclosporin A. *Transplantation 33:31.*
2. **Kilshaw, P. J., L. Brent, and M. Pinto.** 1975. Suppressor T cells in mice made unresponsive to H-2 incompatible skin grafts. *Nature 255:489.*
3. **Marquet, R. L., G. A. Heystek, and J. Tinbergen.** 1971. Specific inhibition of organ allograft rejection by donor blood. *Transplant. Proc. 3:708.*
4. **Hall, B. M.** 1985. Mechanisms maintaining enhancement of allografts. *J. Exp. Med. 161:123.*
5. **Mullen, V., M. Takasugi, and W. H. Hildeman.** 1973. The immunological status of rats with long-surviving (enhanced) kidney allografts. *Transplantation 15:238.*
6. **Hutchinson, I. V.** 1986. Suppressor T cells in allogeneic models. *Transplantation 41:547.*
7. **Sheriff, M. H. R., T. Yayha, and H. A. Lee.** 1978. Is azathioprine necessary in renal transplantation? *Lancet 1:118.*
8. **Zoller, K. M., S. I. Cho, J. J. Cohen, and J. T. Harrington.** 1980. Cessation of immunosuppressive therapy after successful transplantation: a national survey. *Kidney Int. 18:110.*
9. **Goulmy, E., G. Persijn, E. Blokland, J. D'Amaro, and J. J. van Rood.** 1981. Cell-mediated lympholysis studies in renal allograft recipients. *Transplantation 31:210.*
10. **Thomas, J., F. Thomas, and H. M. Lee.** 1977. Why do HLA-nonidentical renal allografts survive 10 years or more? *Transplant. Proc. 9:85.*
11. **Herzog, W., B. Zanker, E. Irschick, C. Huber, H. E. Franz, H. Wagner, and D. Kabelitz.** 1987. Selective reduction of donor-specific cytotoxic T lymphocyte precursors in patients with a well-functioning kidney allograft. *Transplantation 43:384.*
12. **Goulmy, E., E. Blokland, G. Persijn, L. Paul, R. Koene, J. Wilmink, and J. J. van Rood.** 1985. HLA matching regulates post renal transplant CML non-reactivity. *J. Immunol. 135:3082.*
13. **Malissen, B., N. Rabai, A. Liabeuf, and C. Mawas.** 1982. Human cytotoxic T cell structures associated with expression of cytolysis. I. analysis at the clonal level of the cytolysis inhibition effect of 7 monoclonal antibodies. *Eur. J. Immunol. 9:739.*
14. **Rebai, N., and B. Malissen.** 1983. Structural and genetic analysis of HLA class I molecules using xenoantibodies. *Tissue Antigens 22:107.*
15. **Liabeuf, A., C. Le Borgne de Kaouel, F. M. Kourilski, B. Malissen, Y. Manuel, and A. R. Sanderson.** 1981. An antigenic determinant of human $\beta$2-microglobulin masked by the association with HLA heavy chains at the cell surface: analysis using monoclonal antibodies. *J. Immunol. 127:1542.*
16. **Koning, F.** 1984. Identification and functional relevance of epitopes on human lymphocytes. Doctoral dissertation, Leiden University, Leiden, The Netherlands.
17. **Koning, F., J. Raghoubar, G. M. T. Schreuder, R. Schuurman, and H. Bruning.** 1985. A monoclonal antibody detecting an HLA-DQw1 related determinant. *Tissue Antigens 26:100.*
18. **Rebai, N., B. Malissen, M. Pierres, R. S. Acolla, G. Corte, and C. Mawas.** 1983. Distinct HLA-DR epitopes and distinct families of HLA-DR molecules defined by 15 monoclonal antibodies either anti-DR or allo-anti-Ia crossreacting with human DR-molecules. *Eur. J. Immunol. 13:106.*
19. **Spits, H., J. Borst, M. Giphart, J. Coligan, C. Terhorst, and J. E. de Vries.** 1984. HLA-DC antigens can serve as recognition elements for human cytotoxic T lymphocytes. *Eur. J. Immunol. 14:299.*
20. **Borst, J., J. J. M. van Dongen, R. L. H. Bolhuis, P. J. Peters, D. A. Hafler, E. de Vries, and R. J. van der Griend.** 1988. Distinct molecular forms of human T cell receptors $\gamma/\delta$ detected on viable T cells by a monoclonal antibody. *J. Exp. Med. 167:1625.*
21. **Ciccone, E., S. Ferrini, C. Bottini, O. Viale, I. Prigione, G. Pantaleo, G. Tambussi, A. Moretta, and L. Moretta.** 1988. A monoclonal antibody specific for a common determinant of the human T cell receptor $\gamma/\delta$ directly activates $CD3^+WT31^-$ lymphocytes to express their functional program(s). *J. Exp. Med. 168:1.*
22. **Jitsukawa, S., F. Faure, M. Lipinski, F. Triebel, and T. Hercend.** 1987. A novel subset of human lymphocytes with a T cell receptor-$\gamma$ complex. *J. Exp. Med. 166:1192.*
23. **Zhang, L., S. Li, B. A. E. Vandekerckhove, A. Termytelen, J. J. van Rood, and F. H. J. Claas.** 1989. Analysis of cytotoxic T cell precursors against individual HLA-A and -B antigens. *J. Immunol. Methods 121:39.*
24. **Widmer, M. B., R. B. Acres, N. M. Sassenfield, and K. H. Grabstein.** 1987. Regulation of cytolytic cell populations from human peripheral blood by B cell stimulatory factor 1 (interleukin 4). *J. Exp. Med. 166:1447.*
25. **Spits, H., H. Yssel, X. Palliard, R. Kastelijn, C. Figdor, and J. E. de Vries.** 1988. IL-4 inhibits IL-2-mediated induction of human lymphokine-activated killer cells, but not the generation of antigen-specific cytotoxic T lymphocytes in mixed lymphocyte cultures. *J. Immunol. 141:29.*
26. **Koning, F., I. Schreuder, M. Giphart, and H. Bruning.** 1984. A mouse monoclonal antibody detecting a DR-related MT2-like specificity: serology and biochemistry. *Hum. Immunol. 9:221.*
27. **Palliard, X., R. de Waal Malefijt, J. E. de Vries, and H. Spits.** 1988. IL-4 can mediate the induction of CD8 on activated human $CD4^+$ T cell clones. *Nature 335:642.*
28. **Lanier, L. L., J. Ruitenberg, R. L. H. Bolhuis, J. Borst, J. H. Phillips, and R. Testi.** 1988. Structural and serological heterogeneity of $\gamma\delta$ T cell antigen receptor expression in thymus and peripheral blood. *Eur. J. Immunol. 18:1985.*
29. **Hall, B. M.** 1989. Transplantation tolerance: a 1988 perspective. *Transplant. Proc. 21:816.*
30. **Heeg, K., and H. Wagner.** 1985. Analysis of immunological tolerance to major histocompatibility complex antigens. I. High frequencies of tolerogen-specific cytotoxic T lymphocyte precursors in mice neonatally tolerized to class I major histocompatibility complex antigens. *Eur. J. Immunol. 15:25.*
31. **Stockinger, B.** 1984. Cytotoxic T-cell precursors revealed in neonatally tolerant mice. *Proc. Natl. Acad. Sci. USA 81:220.*
32. **de Vries, J. E., H. Yssel, and H. Spits.** 1989. Interplay between the T cell receptor/CD3 complex and CD4 or CD8 in the activation of cytotoxic T lymphocytes. *Immunol. Rev. 109:119.*
33. **MacDonald, H. R., N. Thiernesse, and J. C. Cerottini.** 1981. Inhibition of T cell mediated cytolysis by monoclonal antibodies directed against Lyt.2: heterogeneity at the clonal level. *J. Immunol. 126:1671.*
34. **Bonneville, M., J. F. Moreau, E. Blokland, J. Pool, J. P. Moison, E. Goulmy, and J. P. Soulillou.** 1988. T-Lymphocyte cloning from rejected human kidney allograft. II. Recognition repertoire of alloreactive T-cell clones. *J. Immunol. 141:4187.*
35. **Wood, P. J., P. C. Strome, and J. W. Streilein.** 1987. Characterization of cytotoxic cells in mice rendered neonatally tolerant of MHC alloantigens: evidence for repertoire modification. *J. Immunol. 138:3661.*
36. **Matis, L. A., R. Cron, and J. A. Bluestone.** 1987. Major histocompatibility complex-linked specificity of gamma-delta receptor bearing T lymphocytes. *Nature 330:262.*
37. **Rivas, R., J. Koide, M. L. Clearly, and E. G. Engleman.** 1989. Evidence for involvement of the $\gamma\delta$ T cell antigen receptor in cytotoxicity mediated by human alloantigen-specific T cell clones. *J. Immunol. 142:1840.*