Human T Cells Respond to Mouse Mammary Tumor Virus–encoded Superantigen: Vβ Restriction and Conserved Evolutionary Features

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Summary

Mouse mammary tumor virus (MMTV)-encoded superantigens (SAGs) influence the murine T cell repertoire and stimulate a strong mixed lymphocyte response in vitro. These SAGs are encoded by the open reading frame of the 3′ long terminal repeat of MMTV, termed MMTV SAGs. The T cell response to MMTV SAGs is Vβ restricted and requires expression of the class II molecules of the major histocompatibility complex (MHC) on the presenting cells. While human T cells respond to bacterial SAGs, it is not known if human T cells or human MHC class II molecules can interact with MMTV SAGs. A fibroblastic cell line expressing the human MHC class II molecule HLA-DR1 and the *Mtv-7 sag* gene encoding Mls-1 was used to stimulate human T cells. We show here that human T cells efficiently proliferate in response to Mls-1 presented by HLA-DR1. This T cell response was inhibited by mAbs directed against CD4 or MHC class II molecules but not by mAbs specific for CD8 or MHC class I molecules. Moreover, the response to Mls-1 was limited to human T cells expressing a restricted set of T cell receptor Vβ chains. Human T cells expressing Vβ12, 13, 14, 15, and 23 were selectively amplified after *Mtv-7 sag* stimulation. Interestingly, these human Vβs share the highest degree of homology with the mouse Vβs interacting with Mls-1. These results show a strong evolutionary conservation of the structures required for the presentation and the response to retrovirally encoded endogenous SAGs, raising the possibility that similar elements operate in humans to shape the T cell repertoire.

Superantigens (SAGs)[1] are a new class of Ags that stimulate a large number of human and mouse T cells expressing specific variable regions of the TCR β chain (1–3). The response of T cells to SAG requires the expression of MHC class II molecules on the cell surface of the APCs (1, 4, 5). Unlike nominal antigens, SAGs are not processed into peptides by APCs. Two sets of molecules have been shown to exhibit the properties of SAGs. Bacterial toxins expressed by *Staphylococcus* and *Streptococcus* bacteria stimulate large number of T cells after their interaction with MHC class II molecules. More recently, protein products encoded within the 3′ LTR of mouse mammary tumor virus (MMTV) were shown to be directly associated with Mls SAG reactivity (6–12).

In the mouse, molecules of the Mls loci are directly involved in shaping the TCR repertoire (13–15). Genetic analysis of recombinant mouse strains established a direct correlation between endogenous MMTV integrants and the expression of Mls Ags (6–8). Moreover, exogenous MMTVs were also shown to be directly responsible for the deletion of specific sets of Vβs (9–12). Transfection of class II$^+$ cells with the *sag* gene of endogenous or infectious MMTVs is sufficient to stimulate T cells expressing particular Vβs (16–18). *Mtv-7 sag* encodes Mls-1, which stimulates in vitro and deletes in vivo T cells expressing the murine Vβ6, 7, 8.1, and 9 chains (14, 15, 19).

Retroviruses of the MMTV family are responsible for mammary tumors (20). Recent reports have shown that the SAG encoded by infectious MMTV is directly responsible for mammary tumors (21). Other reports have suggested a role for SAGs in autoimmune diseases and immunodeficiencies both in mouse and in human (22–26). While human T cells readily respond to bacterial SAGs, little is known about their capacity to respond to virally encoded SAGs. One recent report has suggested that the rabies virus nucleoprotein can stimulate human T cells in a Vβ-specific fashion (27). It becomes important to determine whether human MHC class II mole-

[1] *Abbreviations used in this paper:* MMTV, mouse mammary tumor virus; RT, reverse transcriptase; SAG, superantigen.

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cules can present or human TCR β chains can respond to retrovirally encoded SAGs.

To analyze this question, we have used murine fibroblasts expressing the human MHC class II molecule HLA-DR1 and the *Mtv-7 sag* Mls-1 to stimulate human T cells in vitro. Specific stimulation of human T cells bearing a restricted number of Vβ gene segments was shown. Moreover, the TCR Vβs expressed on the human T cells responding to Mls-1 exhibited a strong degree of homology to the mouse Vβs, which are stimulated by Mls-1.

Materials and Methods

Antibodies. mAbs against the human TCR β chain used in this study were S511 (Vβ12.2) (28), 3D11 (Vβ5) (29), Hut-78 (Vβ1) (O. Kanagawa, personal communication), MX6 (Vβ8) (29), OT145 (Vβ6.7a) (30, 31), MKB-1 (Vβ9) (O. Kanagawa, personal communication), C1 (Vβ17) (32) and MH3-2 (Vβ5) (O. Kanagawa, personal communication).

L-243 is an anti-HLA-DR mAb, BL4 and OKT4 are anti-human CD4 mAbs, W6-32 in an anti-human MHC class I mAb, OKT8 is an anti-human CD8, and Leu-4 is a human CD3-specific mAb. Hybridomas were obtained from American Type Culture Collection (Rockville, MD).

Human T Cell Purification. T cells were purified from peripheral blood of DR1$^+$ individuals using rosetting with SRBC (Quelab, Montréal, Québec, Canada). Briefly, peripheral blood was diluted in PBS and underlayered with Ficoll-Hypaque (Pharmacia, LKB Biotechnology AB, Uppsala, Sweden). After centrifugation, the interface was collected and diluted with an equal volume of PBS. Cells were washed and resuspended at 5 × 10^6 cells/ml in PBS. They were then incubated at 1:1 (vol/vol) ratio with FCS (Gibco Laboratories, Grand Island, NY) preabsorbed with SRBC and 2-aminoethylisothiouronium bromide (Sigma Chemical Co., St. Louis, MO) -treated SRBC (1:20) for 10 min at 37°C. Cells were then spun down and incubated for an additional 1 h on ice. The supernatant was aspirated and the pellet was resuspended in 4 ml of RPMI (Gibco Laboratories) supplemented with 10% FCS (Gibco Laboratories). Cells were separated on Ficoll. SRBC within the T cell pellet were lysed with 5 ml of buffer containing 0.15 M NH_4Cl, 9.4 M Na_2CO_3, 0.1 mM EDTA for 2–5 min at room temperature. Cell suspensions were then diluted and washed. The Ficoll interface was kept to be used as a source of autologous feeder cells.

The T cell and non-T cell fractions were stained with anti-CD3 (Leu-4-FITC) and anti-HLA-DR (L-243-bio) mAbs to determine the degree of purity. T cell purity was always >98%, while the non-T cell fraction contained <20% of T cells as confirmed by FACS® analysis (Becton Dickinson & Co., Mountain View, CA).

Transfection of Mtv-7 sag in DAP DR1 Fibroblasts. Fibroblasts expressing HLA-DR1 molecules were transfected using the calcium phosphate precipitation technique (33) with the *Mtv-7 sag* gene cloned into the eukaryotic expression vector pHAβ-Apr1-neo (34). 2 d after transfection, cells were trypsinized and plated at 10^4 cells per well in 24-well plates in the presence of 1 mg/ml G418 (Gibco Laboratories). This selection procedure allowed us to generate individual clones of DR1 cells transfected with *Mtv-7 sag*. Northern blot analysis was used to confirm *Mtv-7 sag* expression. Two clones, 3A5 and 3B2, were selected and used in these experiments.

Human T Cell Stimulation with Mtv-7 sag. Purified human T cells were stimulated with DAP DR1 fibroblasts expressing the cDNA encoding for the 3′ open reading frame (ORF) of *Mtv-7* in the presence or absence of autologous feeder cells. Controls included DR1 cells that had not been transfected with the gene encoding *Mtv-7 sag*. 6 × 10^5 T cells were cultured with mitomycin C (Sigma Chemical Co.) -treated DR1$^+$ or DR1$^+$ *Mtv-7 sag*$^+$ DAP fibroblasts (100 μg/ml of mitomycin C for 10^7 cells, 1 h at 37°C) at different effector/stimulator ratios in 96-well round-bottomed plates. Cultures were carried out at 37°C, 5% CO_2 in complete RPMI supplemented with 10% FCS. Usually, 5 × 10^5 autologous irradiated feeder cells (5,000 rad) were added in each well. After 3 d of coculture, 1 μCi of [^{3}H]Tdr (DuPont Co.-New England Nuclear, Boston, MA) was added for 18 h. Cells were harvested and [^{3}H]Tdr incorporation was determined using a β plate counter (Pharmacia, LKB Biotechnology AB).

mAb Inhibition of Mls-1 Stimulation. 6 × 10^5 T cells were cocultured with 2 × 10^5 mitomycin C-treated DR1$^+$ Mls-1$^+$ 3A5 DAP cells in the presence of 5 × 10^5 autologous irradiated (5,000 rad) feeder cells. mAbs against CD4 (BL-4), CD8 (OKT8), HLA-DR (L-243), or MHC class I (W6-32) were added at 2, 0.2, and 0.02 μg/ml. T cell proliferation was measured after 72 h incubation by [^{3}H]Tdr incorporation as described above. All of the mAbs used in these experiments were purified on protein G columns (Pharmacia, LKB Biotechnology AB).

T Cell Expansion after Mls-1 or PHA Stimulation. Mls-1 stimulation was performed as described in the previous section at an effector/stimulator ratio of 3:1. For PHA stimulation, 10^6 T cells were cultured with 2 × 10^5 irradiated autologous feeder cells and 1 μg/ml PHA (Wellmark Diagnostics, Guelph, Ontario, Canada) in a final volume of 2 ml in 24-well plates. After 3–4 d of stimulation, recombinant human IL-2 (10 U/ml) (Cetus Corp., Emoryville, CA) was added. T cells were expanded in recombinant IL-2 for 10–14 d.

Cytofluorometric Analysis of the Vβ Repertoire after Mls-1 or PHA Stimulation. Two-color flow cytometric analysis was performed, 10 d after stimulation, using CD4- and TCR Vβ-specific mAbs. Briefly, 2.5 × 10^5 cells were first incubated with saturating concentrations of anti-Vβ mAbs for 30 min at 4°C in complete medium. Cells were then washed with PBS and stained using a 1:100 dilution of goat anti-mouse Ig coupled to PE (GAMPE; Southern Biotechnology Associates, Inc., Birmingham, AL). Cells were incubated for 30 min at 4°C and washed in PBS. Normal mouse serum was then added for 15 min at 4°C to block the GAMPE antibodies. Cells were washed and counterstained with FITC-conjugated anti-CD4 mAb (OKT4-FITC, 1/200 dilution) for 30 min at 4°C. Stainings were analyzed on a FACScan® (Becton Dickinson & Co.) using two-color cytofluorometry. 10^4 live cells were gated using forward and 90° light scatter. Results are expressed on a four-decade logarithmic scale for each two-color histogram.

PCR Primers. PCR primers were synthesized on an oligonucleotide synthesizer (Applied Biosystem Canada Inc., Mississauga, Ontario, Canada). Primer sequences of the TCR Vβ-specific oligonucleotides are listed in Fig. 1. The G-C content was ~40–60%. The specificity of the different Vβ oligonucleotides was determined by crosshybridization. The oligonucleotide sequences of the primers for the TCR α and β chain constant regions are: 3′ Cβ, 5′ TCT ACC CCA GGC CTC GGC GCT GAC GAT 3′; 3′ Cα, 5′ AGC CGC AGC GTC ATG AGC AGA TTA AAC CCG 3′; 5′ Cα, 5′ GCA TGT GCA AAC GCC TTC AAC AAC AGC 3′.

PCR Analysis of the Expressed Vβs Repertoire. T cells were pelleted for RNA extraction 10 d after stimulation and expansion in recombinant IL-2 (Cetus Corp.). Total RNA was prepared using RNAzol (Cinna-Biotecx, Houston, TX). 10 μg of total RNA was boiled 2–5 min and used to synthesize the first-strand cDNA by adding 5 μg of random hexamer oligonucleotides and 60 U of Moloney leukemia virus reverse transcriptase (RT) (Life Sciences,

Inc., St. Petersburg, FL). The RT reaction was performed in the presence of RNAse inhibitor, 1 μM dNTPs (Pharmacia, LKB Biotechnology AB) in a buffer containing 10 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, and 40 mM KCl at 42°C for 45 min. RT and nucleotides were then added and followed by a second incubation period of 45 min. The RT reaction was stopped by heating the mixture at 65°C for 10 min. The cDNA preparation was precipitated and used for PCR Vβ analysis. The different Vβs were amplified using a 5′ Vβ-specific primer and a common 3′ Cβ primer. 5′ and 3′ Cα primers were included in each reaction tube as internal control. The 3′ Cβ and 3′ Cα primers were radiolabeled with γ-[^{32}P]ATP (30,000 Ci/mmol); 50 pmol of oligonucleotide was incubated with 4 μl of γ-[^{32}P]ATP and 20 U of T4 polynucleotide kinase (Pharmacia, LKB Biotechnology AB) in the manufacturer's buffer for 1 h at 37°C. Free nucleotides were eliminated using a G-25 superfine Sephadex column. PCR amplification was performed with 25 pmol of 5′ Vβ, 5′ Cα, and a mixture of cold and radiolabeled 3′ Cβ (22 pmol of cold and 3 pmol of radiolabeled oligonucleotide) and 3′ Cα (25 pmol of cold and 0.3 pmol of radiolabeled oligonucleotide). The PCR reaction was carried out using 200 μM dNTPs, 2 U of Taq polymerase (Perkin-Elmer Corp., Norwalk, CT) in 1× Taq buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% [wt/vol] gelatin) in a final volume of 100 μl. Amplification was performed for 30 s at 94°C, 45 s at 55°C, and 60 s at 72°C for 25 cycles. Half the volume of each PCR reaction was loaded and separated on a 10% PAGE containing 7 M urea. Gels were exposed overnight on PhosphorImager screens (Molecular Dynamics, Inc., Sunnyvale, CA). Quantitation of the radioactive signal for each Vβ was carried out using the Imagequant software and the PhosphorImager (Molecular Dynamics, Inc.).

Name	Sequence	Specificity
Vβ 1	5′ AGA TTC TGG AGT CAC ACA AAC CCC AAA GCA C 3′	Vβ 1.1, Vβ 1.2
Vβ 2	5′ GAG CTG GGT TAT CTG TAA GAG 3′	Vβ 2.1
Vβ 3	5′ AGA TGT GAA AGT AAC CCA GAG CTC GAG ATA T 3′	Vβ 3.1, Vβ 3.2
Vβ 4	5′ CAA GTC GAT AGC CAA GTC ACC ATG 3′	Vβ 4.
Vβ 5.1	5′ CTC CCC TAT CTC TGG GCA TAG AG 3′	Vβ 5.1, Vβ 5.4
Vβ 5.2	5′ CTC TCC TAA GTC TGG GCA TGA 3′	Vβ 5.2
Vβ 6	5′ AGG TGC TGG AGT CTC CCA GAC CCC CAG TA 3′	Vβ 6.1, Vβ 6.3
Vβ 7	5′ CAT GGG AAT GAC AAA TAA GAA GTC 3′	Vβ 7.1
Vβ 8	5′ AGA TGC TGG AGT TAT CCA GTC ACC CCG CCA TGA 3′	Vβ 8.1, Vβ 8.3
Vβ 9	5′ AAC GAC AAG TCC ATT AAA 3′	Vβ 9.1
Vβ10	5′ AGA CAC CAA GGT CAC CCA GAG ACC TAG ACT T 3′	Vβ10.1, Vβ10.2
Vβ11	5′ AGA AGC TGA CAT CTA CCA GAC CCC AAG ATA C 3′	Vβ11.1
Vβ12	5′ AAA TGC TGG TGT CAC TCA GAC CCC AAA ATT C 3′	Vβ12.3
Vβ13	5′ GTT GGT GCT GGT ATC ACT GA 3′	Vβ13.1
Vβ14	5′ AGT TAA CAG TGA CTT GTT CTC AGA A 3′	Vβ14.1
Vβ15	5′ AGA TGC TGA TGT TAC CCA GAC CCC AAG GAA 3′	Vβ15.1
Vβ16	5′ GAC TGT GAC TCT GAG ATG TGA C 3′	Vβ16.1
Vβ17	5′ AGA TGG TGG AAT CAC TCA GTC CCC AAA GTA C 3′	Vβ17.1
Vβ17b	5′ ACC CAA GAC ACC TGG TCA GGA GGA GG 3′	Vβ17.1
Vβ18	5′ AAA TGC CGG CGT CAT GCA GAA CCC AAG ACA C 3′	Vβ18.1
Vβ19	5′ ACA TGC CAA AGT CAC ACA GAC TCC AGG ACA TTT 3′	Vβ19.1
Vβ20	5′ ACA TGC CAA AGT CAC ACA GAC TCC AGG ACA TTT 3′	Vβ20.1
Vβ21	5′ GAG TGT GGC TTT TTG GTG CAA 3′	Vβ21.1*
Vβ22	5′ TGG GAC AGG AAG TCA TCT TGC 3′	Vβ22.1*
Vβ23	5′ GAC AGC TGA TCA AAG AAA AGA 3′	Vβ23.1*
Vβ24	5′ AGG TTA CCC AGT TTG GAA AGC 3′	Vβ24.1*

Figure 1. Description and sequence of human TCR Vβ-specific oligonucleotide primers. *From reference 44.

Results

Mls-1-expressed DR1$^+$ DAP Fibroblasts Stimulate the Proliferation of Human T Cells. To determine whether human T cells are able to respond to Mtv-7 SAG Mls-1, the *Mtv-7 sag* gene was transfected into DR1$^+$ DAP fibroblasts. Expression of *Mtv-7 sag* transcripts was confirmed by Northern blot analysis (Subramanyam, M., B. McLellan, N. Labrecque, R.-P. Sekaly, and B. T. Huber, manuscript submitted for publication). The human MHC class II molecule HLA-DR1 efficiently presented the Mtv-7 SAG to murine T cell hybridomas expressing the appropriate TCR Vβs (Vβ6 and 8.1) (Subramanyam, M., et al., manuscript submitted for publication). These DAP cells were then used to stimulate purified populations of human T cells obtained from HLA-DR1$^+$ individuals. Fig. 2 *A* shows a 30-fold increase in [^{3}H]Tdr incorporation 72 h after the addition of purified human T cells to mitomycin C–treated DR1$^+$ Mls-1$^+$ DAP transfectants. T cells cultured in the presence of DAP cells expressing only DR1 did not show such an increase. Proliferation, after 72 h of coculture, was dependent on the number of DR1$^+$ Mls-1$^+$ APCs. Significant proliferation, fivefold over DAP DR1 stimulation, was still observed at the lowest effector/stimulator cell ratio tested (10:1).

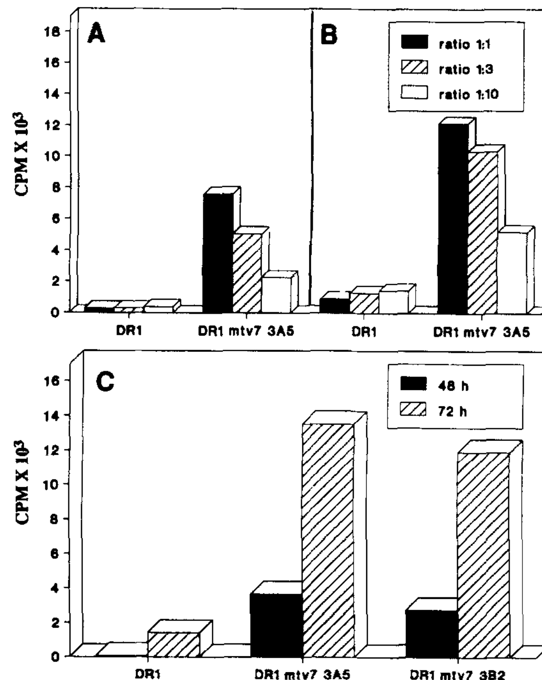

Figure 2. Stimulation of human purified T cells by DR1$^+$ Mls-1$^+$ DAP cells. (*A* and *B*) Proliferative response of human T cells stimulated with DR1$^+$ (DAP cells transfected with HLA-DR1) and DR1$^+$ Mls-1$^+$ 3A5 (DAP cells transfected with HLA-DR1 as well as *mtv-7 sag* gene). Stimulation was performed in the presence (*A*) or absence (*B*) of 10^5 irradiated autologous feeder cells at different effector/stimulator ratios. (*C*) Kinetics of human T cell response to Mls-1. Proliferative response of human T cells stimulated with DR1$^+$ alone, DR1$^+$ Mls-1$^+$ 3A5 (DR1 *mtv-7* 3A5), or DR1$^+$ Mls-1$^+$ 3B2 (DR1 *mtv-7* 3B2) DAP cells (effector/stimulator ratio, 1:3) in the presence of 10^5 irradiated autologous feeder cells, measured after 48 and 72 h. Bars represent the mean of triplicate values of [^{3}H]Tdr incorporation.

DAP cells transfected with class II molecules are poor stimulator cells for both allogeneic and xenogeneic responses (35). For this purpose, human autologous irradiated non-T cells were added to the coculture, which increased human T cell proliferation by at least twofold (see Fig. 2 *B*). The kinetics of the human T cell response to Mls-1 are comparable to what is observed when the same human T cells are stimulated with bacterial SAGs (data not shown). After 48 h of stimulation the T cell response was very low (fivefold), however, a considerable increase (30-fold) is observed by 72 h (Fig. 2 *C*). Similar kinetics were observed when two different DR1$^+$ Mls-1$^+$ DAP transfectants were used (Fig. 2 *C*).

Presentation of Mtv-7 SAG to Human T Cells Is Inhibited by Anti-CD4 and Anti-HLA-DR but Not by Anti-CD8 and Anti-MHC Class I mAbs. In the murine system, the Mls response is blocked by antibodies directed to MHC class II molecules (36, 37). To further confirm the similarity of the human T cell response to Mls-1, mAb inhibition experiments were performed. Anti-HLA-DR mAb (L-243) completely inhibited T cell proliferation. This inhibition occurred in a dose-dependent fashion, as shown in Fig. 3, confirming the requirement for MHC class II molecules for an Mtv-7 SAG–specific response. Murine T cells responding to Mls-1 are mostly CD4$^+$, and CD4-specific mAbs significantly inhibit this response (5). The human T cell response to DR1$^+$ Mls-1$^+$ cells was inhibited in a dose-dependent manner when anti-CD4 (BL-4) mAb was added to the culture (see Fig. 3). However, T cell proliferation was still observed (fourfold over background) at the highest concentration of CD4-specific mAb (2 μg/ml), suggesting that CD4$^-$ T cells were also responding to Mls-1. Recent reports have demonstrated that murine CD8$^+$ T cells can also proliferate in response to Mls-1 (38, 39). Interestingly, anti-CD8 (OKT8) mAb does not affect the T cell response (Fig. 3), as was previously shown to be the case for murine CD8$^+$ T cells responding to Mls-1 (H. R. McDonald, personal communication). As predicted, anti-MHC class I (W6-32) mAb had no effect on Mls-1 stimulation of human T cells (Fig. 3). These results further confirm the parallel between human and murine T cell responses to retroviral SAGs.

Mls-1 Presentation by DR1$^+$ DAP Fibroblasts to Human T Cells Increases the Number of TCR Vβ12–expressing T Cells. The T cell response to bacterial and retroviral SAGs is restricted to T cells expressing only specific sets of Vβ regions. To confirm that the response of human T cells to *Mtv-7 sag* was characteristic of a SAG response, human T cells responding to Mls-1 presented by DR1$^+$ DAP were stained with a panel of TCR Vβ–specific mAbs. Human T cells were purified from peripheral blood of DR1$^+$ individuals to avoid an allogeneic response. Figs. 4 and 5 represent the results of the cytofluorometric analysis of the Vβ usage after stimulation with PHA, DR1$^+$ DAP, DR1$^+$ Mls-1$^+$ 3A5 DAP, and

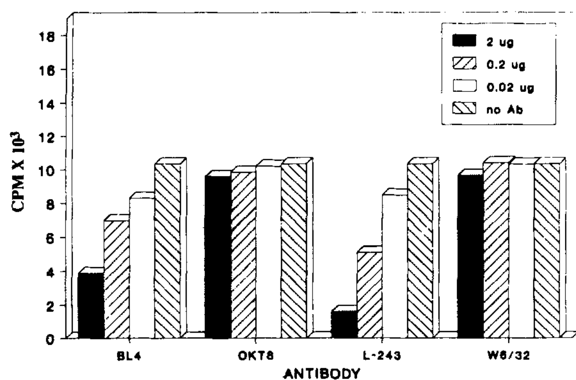

Figure 3. mAb inhibition of Mls-1 stimulation. Mls-1 stimulation of human T cells is inhibited by anti-CD4 and anti-MHC class II mAbs, but not by anti-CD8 and anti-MHC class I mAbs. T cells were stimulated with DR1$^+$ Mls-1$^+$ 3A5 DAP cells at an effector/stimulator ratio of 1:3 in the presence or absence of anti-CD4 (BL4) anti-CD8 (OKT8)-, anti-HLA-DR (L-243)-, or anti-MHC class I (W6-32)-specific mAbs. mAb concentrations ranged between 0.02 and 2 μg/ml. Bars represent the mean of triplicate values of [^{3}H]Tdr incorporation.

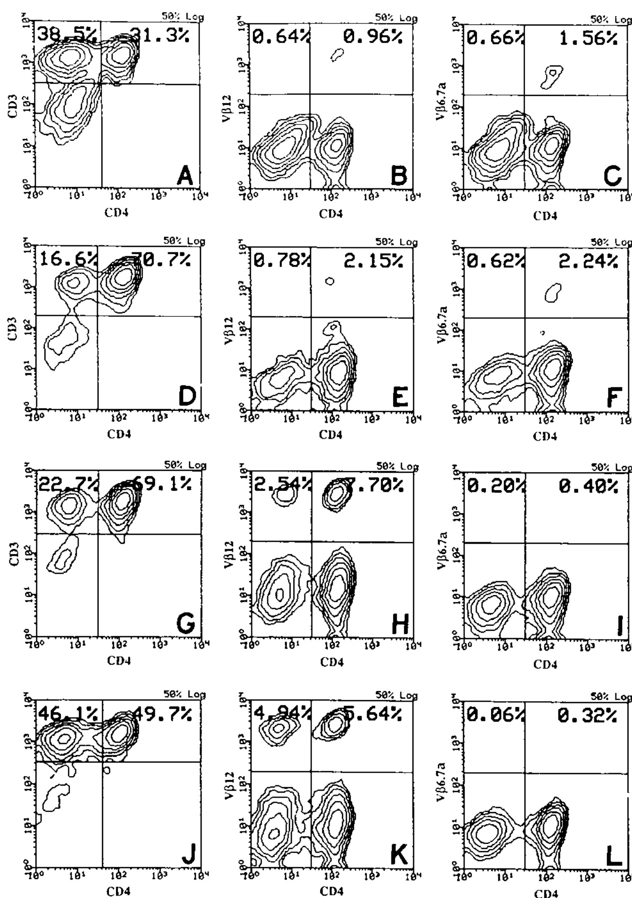

Figure 4. Cytofluorometric analysis of TCR Vβ usage by human T cells in response to Mls-1 presented by DR1$^+$ DAP APCs. T cells were stained with a panel of human Vβ-specific mAbs, followed by goat anti–mouse Ig antiserum (PE conjugated), and then counterstained with an anti-CD4-specific mAb (FITC conjugated) (OKT4). For simplification only contour graphs for CD3, Vβ12, and Vβ6.7a are shown. Contour graphs represent Vβ expression on CD4$^+$ and CD4$^-$ after PHA (*A–C*), DR1$^+$ (*D–F*), DR1$^+$ Mls-1$^+$ 3A5 (*G–I*), and DR1$^+$ Mls-1$^+$ 3B2 stimulation (*J–L*). The percentage of CD4$^+$ (*top right*) and CD4$^-$ (*top left*) are indicated. 10^4 viable cells, gated by light scatter, were accumulated for each contour graph. Results are illustrated on a four-decade logarithmic scale.

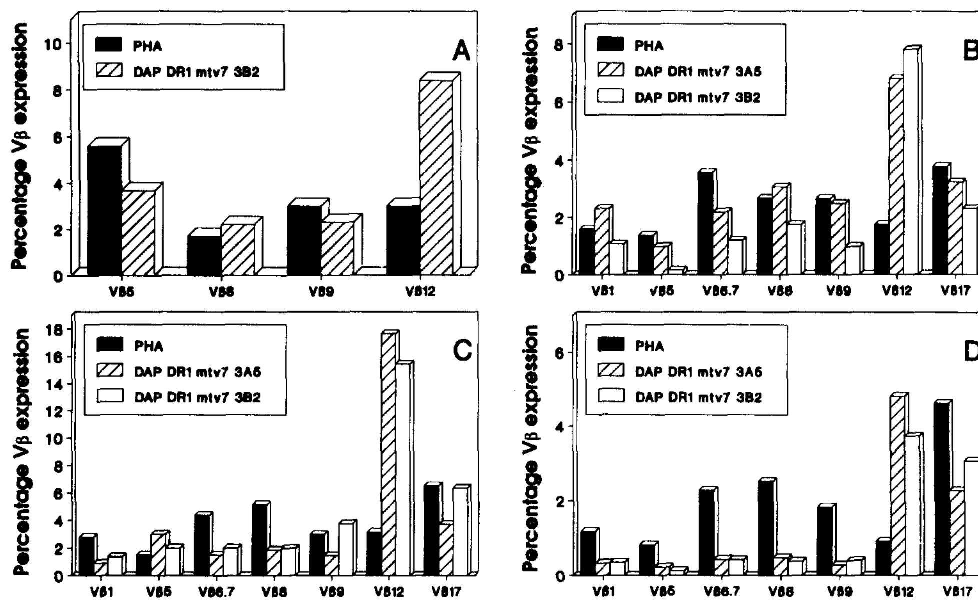

Figure 5. Selective expansion of human T cells expressing the TCR Vβ12 region after Mls-1 stimulation. T cells stimulated with PHA or Mls-1 were kept in culture for 10–14 d in the presence of IL-2 and stained with the various Vβ-specific mAbs, as described in Fig. 4. Percentages of T cells expressing each Vβ in four different experiments are shown. The HLA type of the individuals was: A1, w33; B14, w55; Cw3, w8, DR1,-;-; DQw1; A32, 33; B14, 35; Cw4, w8, DR1, 4; w53; DQw1, w3; A1, 3; B7, 13; Cw6, w7; DR1, 7, w53, DQw1, w2.

$DR1^+$ $Mls\text{-}1^+$ 3B2 DAP. A similar profile in Vβ usage was observed when T cells were stimulated with PHA (Fig. 4, *A–C*) or with $DR1^+$ fibroblasts (Fig. 4, *D–F*). For simplification only the CD3, Vβ12, and Vβ6.7 stainings are shown in Fig. 4. Most if not all the Vβs were expressed at similar levels when purified T cells were stimulated with PHA or cocultured with DAP DR1 cells. Moreover, there was little difference in the Vβ profile in PHA-stimulated cells before IL-2 expansion (T cells were stained with the same mAbs 36 h after PHA stimulation) and after 10–14 d after expansion with IL-2 (data not shown). In contrast, flow cytometric analysis of Vβs expressed in human T cells stimulated with two independent clones of $DR1^+$ $Mls\text{-}1^+$ DAP transfectants (Fig. 4, *G–I* and *J–L*) showed a significant increase in $CD4^+$ $V\beta12^+$ cells and in $CD4^-V\beta12^+$ cells (10-fold). This increase in Vβ12-expressing cells was accompanied by a decrease in the representation of the other TCR Vβs analyzed (Fig. 4 and 5).

This selective expansion of human T cells expressing TCR Vβ12 was reproducible in four separate experiments using two different clones of $DR1^+$ $Mls\text{-}1^+$ DAP transfectants (see Fig. 5). The increase in Vβ12-expressing T cells ranged between 3- and 10-fold in these experiments. There was no detectable increase in any of the other Vβs analyzed in these four experiments, strongly confirming the specificity of the Vβ12 response. Fig. 6 illustrates the number of $V\beta12^+$ $CD4^+$ and $V\beta12^+CD4^-$ cells responding to the two independently derived $DR1^+$ $Mls\text{-}1^+$ DAP transfectants. Both $CD4^+$ and $CD4^-$ $V\beta12^+$ cells proliferated to the $DR1^+$ $Mls\text{-}1^+$ clones (Fig. 6), confirming previous results in the mouse. In three separate experiments the increase in $CD4^+$ T cells was always higher than the one observed in $CD4^-$ T cells. This increase in $CD4^-$ T cells was never greater than sixfold, while as previously mentioned, $CD4^+V\beta12^+$ cells were increased by up to 10-fold.

This relative increase in $V\beta12^+$ cells was also accompanied by a highly significant increase in the absolute number of T cells. Hence, while T cells cocultured together with $DR1^+$ DAP cells and IL-2 proliferated marginally (threefold increase in cell number), there was a reproducible and strong proliferation to both $DR1^+$ $Mls\text{-}1^+$ transfectants (10–33-fold increase in cell number). This increase in cell number was almost parallel to the one observed in T cells stimulated with PHA (10–35 fold).

Selected Vβs Are Amplified after Mls-1 Stimulation as Determined by PCR Analysis of the TCR Repertoire. Flow cytometric analysis indicated that >90% of T cells responding to $DR1^+$ $Mls\text{-}1^+$ fibroblasts in IL-2-expanded cultures expressed the CD3 molecule on their surface. Since the total sum of Vβs expressed, as determined by flow cytometry, did not reflect the number of $CD3^+$ cells observed, this suggested that other Vβs are responding to Mls-1. To further characterize the Vβs expressed by the T cells responding to $DR1^+$ $Mls\text{-}1^+$ DAP cells, a quantitative PCR analysis of the

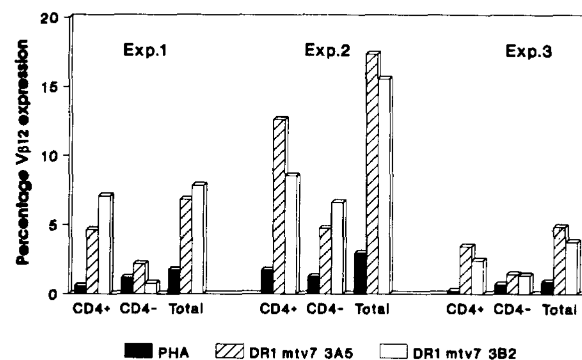

Figure 6. $CD4^+$ and $CD4^-$ Vβ12 T cells respond to $DR1^+$ $Mls\text{-}1^+$ DAP cells. The percentage of total $V\beta12^+$, $CD4^+$ $V\beta12^+$, and $CD4^-$ $V\beta12^+$ T cells obtained in three different experiments after PHA and Mls-1 stimulation was determined by cytofluorometric analysis as described in Fig. 4. Histogram of 10^4 live cells was obtained on a FACscan®.

whole Vβ repertoire was performed. Oligonucleotides specific for the 24 known Vβ families were used to analyze the expressed Vβ repertoire (see Fig. 1). This assay was carried out on PHA-stimulated T cells and on T cells cocultured with the two independently derived DR1$^+$ Mls-1$^+$ transfectants. Fig. 7 *A* shows the PCR Vβ profile of T cells stimulated with PHA and IL-2. Most of the Vβs are expressed. A different picture emerges from the analysis of the Vβ repertoire of T cells responding to the DR1$^+$ Mls-1$^+$ DAP clones, 3A5 (Fig. 7 *B*) and 3B2 (Fig. 7 *C*). Autoradiograms of the quantitative PCR analysis clearly show a strong amplification of a restricted number of Vβs (Vβ12, 13, 14, 15, 16, and 23) only in samples of T cells, stimulated with DR1$^+$ Mls-1$^+$. Most of the other Vβs, which were present in PHA-stimulated cells, were not amplified, demonstrating the preferential and specific expansion of T cells expressing the above-mentioned Vβs. Quantitative PCR analysis confirms results illustrated in Figs. 4–6, showing the increase in relative numbers of Vβ12$^+$ cells. A 3–10-fold increase in the relative expression of the above Vβs was noted after coculture with DR1$^+$ Mls-1$^+$ cells.

Amplification of Vβ12, 13, 14, 15, and 23 was consistently seen in three different experiments using human T cells purified from three different DR1$^+$ donors (see Fig. 8, *B*, *D*, and *F*) when compared with PHA (Fig. 8, *A*, *C*, and *E*). A 3–10-fold increase in the relative expression of the different Vβs was noted in three different individuals tested. Moreover, the two DR1$^+$ Mls-1$^+$ DAP clones reproducibly yielded a selective amplification of Vβ12, 13, 14, 15, and 23 (data not shown).

Discussion

In this report, we demonstrate specific stimulation of human T cells to the *Mtv-7 sag* presented by DR1$^+$ DAP fibroblasts. This T cell stimulation was due to Mls-1 recognition since no T cell proliferation was seen in response to untransfected DR1$^+$ fibroblasts. T cell responses were enhanced by the presence of autologous feeder cells. These cells may provide additional cytokines that help T cell responses. The response of human T cells bears all the characteristics of an Mls-1 stimulation (1); it is inhibited by anti-CD4 and anti–class II mAbs but not by anti-CD8 and anti–class I mAbs. Both CD4$^+$ and CD4$^-$ T cells respond to DR1$^+$ Mls-1$^+$ DAP cells; however, unlike antigen-specific responses, proliferation of both T cell subsets is inhibited by DR-specific mAbs, while mAbs to CD8 and to MHC class I do not have an effect. Similar observations have already been described in human and murine T cell responses to bacterial SAGs (1, 40). The strong primary proliferative response of the human T cells to DR1$^+$ Mls-1$^+$ cells further confirms the parallel with a SAG response.

The hallmark of the response to a SAG is the TCR Vβ restriction of the responding T cells. Indeed, the human T cell response to Mtv-7 SAG presented by the human MHC class II molecules, HLA-DR1, is restricted to T cells expressing a limited set of Vβs, further confirming the parallel between human and murine T cell responses to this retrovirally encoded SAG. Cytofluorometric analysis, using a restricted set of TCR Vβ–specific mAbs, has enabled us to demonstrate that CD4$^+$ Vβ12$^+$ and CD4$^-$ Vβ12$^+$ cells respond to DR1$^+$ Mls-1$^+$ cells. Quantitative PCR analysis of the TCR

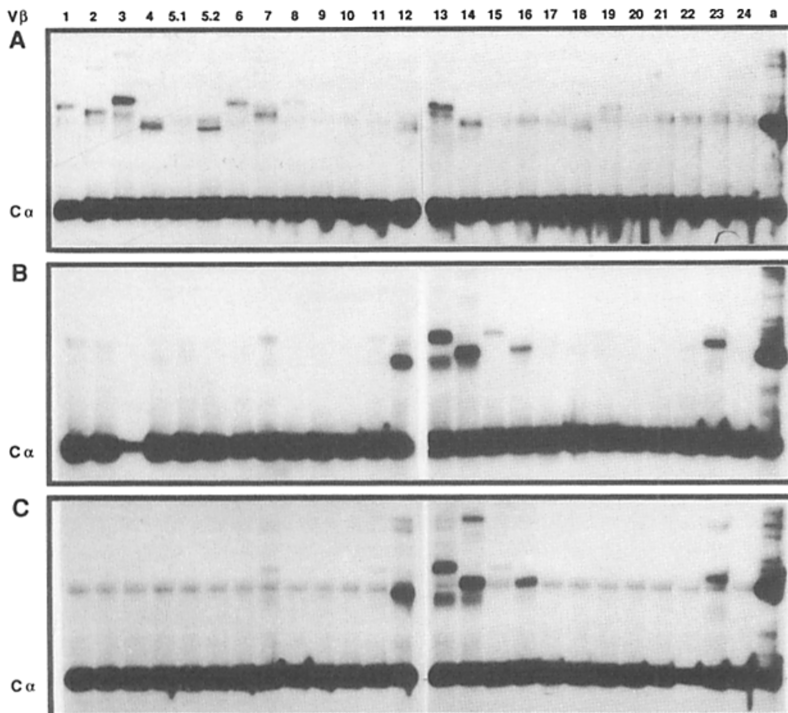

Figure 7. PCR analysis of the TCR Vβ usage of human T cells in response to *Mtv-7 sag* transfected in DR1$^+$ DAP fibroblasts. Electrophoresis of the PCR Vβ reactions of human T cells stimulated with: (*A*) PHA; (*B*) DR1$^+$ Mls-1$^+$ 3A5 DAP cells, and (*C*) DR1$^+$ Mls-1$^+$ 3B2 DAP cells. Each lane represents the amplification of a family of a particular TCR Vβ gene, using a specific oligonucleotide in 5′ and a ^{32}P-labeled TCR-β constant region oligonucleotide in 3′. The TCR Cα was amplified in each reaction as an internal control. Gels were exposed for 2 d. These autoradiograms are representative of three separate experiments.

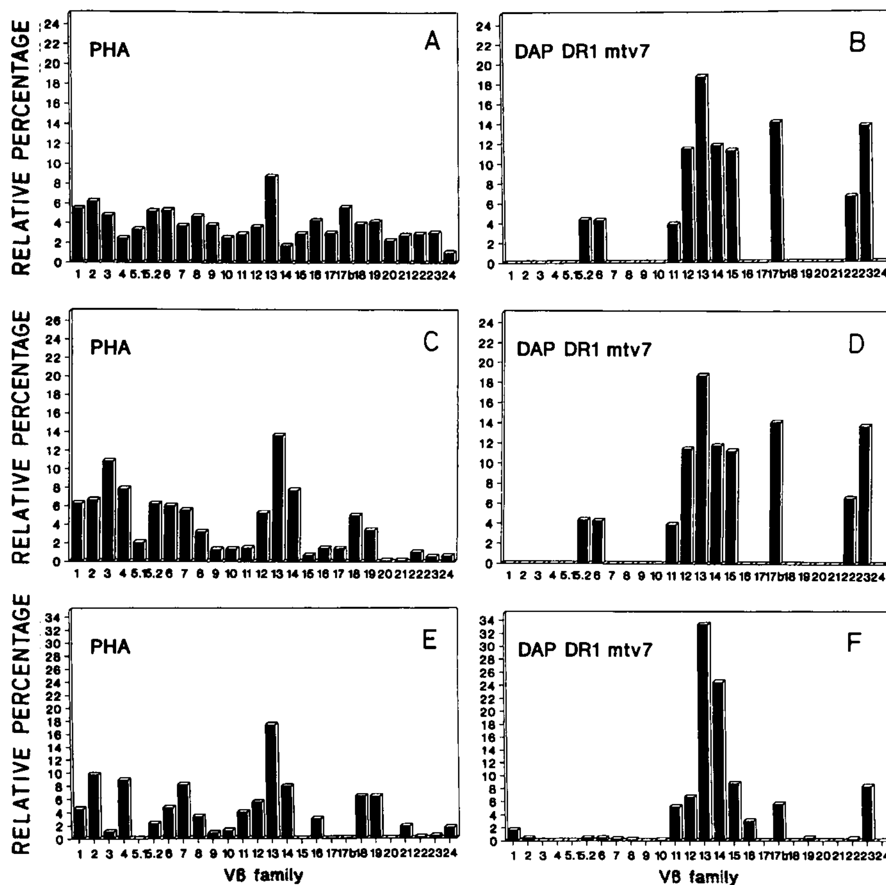

Figure 8. Relative percentage of TCR Vβ usage as determined by PCR analysis. PCR Vβ reactions were subjected to electrophoresis and exposed on a PhosphorImager screen overnight. Radioactive signals were quantitated using a PhosphorImager. The raw PCR value is represented as ratio of the numerical value of the Vβ to the Cα signal (100×). The sum of raw PCR values for each Vβ/Cα combination was determined and used to calculate the relative percent of each Vβ. Represented is the relative percent of TCR Vβ usage after stimulation with PHA (*A*, *C*, and *E*) or DR1^{+} Mls-1^{+} 3B2 DAP (*B*, *D*, and *F*). *A* and *B*, *C* and *D*, and *E* and *F* represent pairwise quantitative PCR values of PHA- and Mls-1-stimulated cells from three different individuals.

repertoire confirmed that Vβ12 cells were expanded in T cells stimulated by DR1^{+} Mls-1^{+} cells. Moreover, other Vβs were also significantly expanded, i.e., Vβ13, 14, 15, and 23. As previously mentioned, the expansion ranged from 3- to 10-fold and was reproducible in three different individuals irrespective of their MHC haplotype. Interestingly, Vβ12 and Vβ17 were amplified by PCR only in two of the three individuals tested. Since the Vβ17 family contains only one member, it is possible that polymorphisms in Vβ17 genes analogous to the polymorphisms previously described for the murine Vβ8 and Vβ17 genes (41, 42) confer to some individuals the capacity to respond to Mls-1.

The human TCR Vβ gene products that recognize Mls-1 share the highest homology with the mouse Vβ chains known to be selectively expanded in Mls-1 stimulation (Vβ6, 7, 8.1, and 9). The human Vβ12 and 13 genes share ~70% homology in nucleotide sequences with the mouse Vβ8.1 gene, while the human Vβ15 gene is 70% homologous to the mouse Vβ7 gene (43). Only the human Vβ23 gene product does not possess strong homology (44–56%) with Mls-1-reactive murine Vβs (44). The homology of the human Vβs responding to Mls-1 with other murine Vβs that do not respond to Mls-1 varies between 40 and 55% (43).

The CDR4 of the TCR Vβ chain encompasses critical residues for the Mls-1 response (42, 45). Residues 68–76 of the CDR4 loop are predicted to lie on a β-pleated sheet away from the CDR1–3, which are involved in conventional antigen recognition. Critical residues for Mls-1 recognition include amino acids 73 and 74, as shown from Vβ polymorphisms in wild mice and by site-directed mutagenesis studies (41, 42, 45). Moreover, residue N74 in an Mls-1-nonreactive Vβ chain carries a potential glycosylation site. Interestingly, none of the Mls-1-reactive human Vβs have potential glycosylation sites in the CDR4. A consensus was derived that clearly shows that two residues, S68 and R69, are conserved in 13 of 14 Mls-1-reactive Vβs. A third highly conserved amino acid (F75) is present in almost all other Vβ sequences. In contrast, only 7 of 38 Mls-1-nonreactive Vβs bear these residues. The human Vβ23, which in our experiments responds very efficiently to DR1 *Mtv-7 sag* fibroblasts, does not carry these two residues in its CDR4 sequence. Since only one Vβ23 sequence is published so far, it is likely that polymorphisms in this Vβ gene could modify the reported Vβ23 sequence and conferring reactivity of Vβ23 to Mls-1.

The results presented here clearly indicate that human T cells carry all the structural features required for an efficient response to murine retrovirally endogenous encoded SAGs. Moreover, this response involves similar mechanisms to those required for the response of murine T cells to Mls-1. The ability of human T cells to respond to the Mtv-7 SAG and the efficient presentation of this SAG by human MHC class II molecules suggest the possibility that the human genome may contain such SAGs. Several reports have suggested polymorphisms within the human Vβ locus at the genomic level

using Southern blots and Vβ-specific probes (30, 46, 47). Some of these polymorphisms have been associated with disease (48). Using human T cell stimulation with Mls-1, it will be interesting to verify if the presence of polymorphisms or lack thereof in the coding sequence of Vβs is correlated with the response to viral SAGs. Such a demonstration would provide functional evidence for the role of polymorphisms within the TCR Vβ genes in the deletion of self-reactive Vβs or in the association of specific sets of TCR Vβs with autoimmune diseases. Indeed, Vβ14 has been associated in one study with rheumatoid arthritis (22), while other groups have failed to observe such a correlation (49–51). It is possible that polymorphisms in the Vβ14 gene could confer to certain individuals the capacity to respond to SAGs encoded by pathogens involved in rheumatoid arthritis. Our experiments provide a model that could be used to identify such functional polymorphisms in TCR Vβ chains.

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