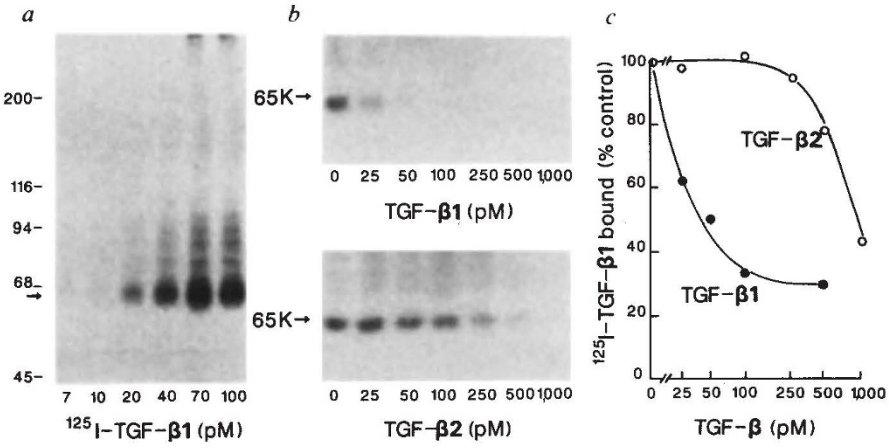

Fig. 3 TGF-β receptors in B6SUtA cells. *a*, Autoradiogram from a SDS polyacrylamide electrophoresis gel containing samples from B6SUtA cells affinity labelled with various concentrations of human ^{125}I-labelled TGF-β1. Affinity labelling was performed by crosslinking 2×10^6 cells in 1 ml of binding buffer with the receptor-bound radioligand using disuccinimidyl suberate as previously described[23]. Arrow, prominent 65 K affinity-labelled species; markers, M_r in thousand. *b*, Autoradiograms from gels containing samples from cells affinity labelled with 25 pM ^{125}I-labelled TGF-β1 alone or with the indicated concentrations of TGF-β1 or TGF-β2. *c*, Binding competition curves obtained with B6SUtA cells incubated with 25 pM ^{125}I-labelled TGF-β1 alone or with increasing concentrations of TGF-β1 or TGF-β2. Binding assays using cell suspensions were done at 0-4 °C as previously described[13,23].

and the heterodimer, TGF-β1.2)[3] in conjunction with the discriminating ligand binding properties of TGF-β receptor types I and II may provide the opportunity to influence selectively certain cellular functions but not others. *In vivo* TGF-β2, from endogenous sources or exogenously administered, may mimic potently only those actions of TGF-β1 that are exerted through receptors shared by both factors, whereas it may act as a poor agonist on systems which, like haematopoietic progenitors, are regulated by receptors that bind TGF-β1 preferentially.

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T-cell receptors of human suppressor cells

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Cells which can suppress the immune response to an antigen (T_S cells) appear to be essential for regulation of the immune system[1,2]. But the characterization of the T_S lineage has not been extensive and many are sceptical of studies using uncloned or hybrid T-cell lines. The nature of the antigen receptor on these cells is unclear. T cells of the helper or cytotoxic lineages appear to recognize their targets using the T-cell receptor (TCR) $\alpha\beta$–CD3 complex[3–5]. TCR β-gene rearrangements are also found in some murine and human suppressor cell lines[6–10] but others have been shown not to rearrange or express the β-chain[11–13] or α-chain[14] genes. We previously established T_S clones derived from lepromatous leprosy patients[15,16] which carry the CD8 antigen and recognize antigen in the context of the major histocompatibility complex (MHC) class II molecules *in vitro*[16]. We here report the characterization of additional MHC-restricted T_S clones which rearrange TCR β genes, express messenger RNA for the α and β chains of the TCR and express clonally unique CD3-associated TCR $\alpha\beta$ structures on their cell surface but do not express the γ chain of the $\gamma\delta$ TCR on the cell surface. We conclude that antigen recognition by at least some human $CD8^+$ suppressor cells is likely to be mediated by TCR $\alpha\beta$ heterodimers.

Leprosy provides an extraordinary model for investigating immunoregulatory mechanisms in man[17,18]. The disease exhibits a clinical spectrum in which the stage of disease can be correlated with the cellular immune responses of the patients to antigens of *Mycobacterium leprae*. In lepromatous leprosy, patients exhibit a selective and specific unresponsiveness to *M. leprae* antigens. We have suggested that this unresponsiveness may partly result from T cells that recognize *M. leprae*-specific antigens or epitopes and block development of potentially beneficial responses of T_H cells *in vivo*[19]. Such *M. leprae*-specific T_S cells have been found in the blood of lepromatous patients and characterized as $CD3^+CD8^+DR^+$ and FcR^+ (refs 20, 21). Using CD8 cells cloned directly from skin lesions of lepromatous patients and the recently developed antibody reagents against framework determinants on human TCR α, β and γ polypeptides[22–26], it became possible directly to examine the antigen receptors of human T_S cells in molecular terms.

T_S clones were derived from skin biopsies of lepromatous

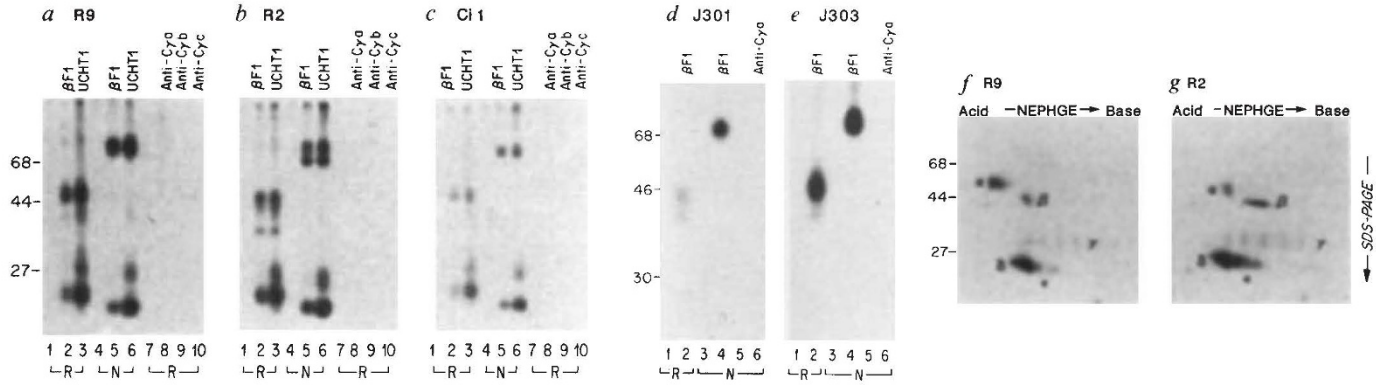

Fig. 1 Immunoprecipitation of T-cell receptors from T_S clones. ^{125}I-labelled R9 (*a*), R2 (*b*), Ci1 (*c*), J301 (*d*) and J303 (*e*) lymphocytes were detergent solubilized and immunoprecipitated with anti-CD3 or anti-TCR antibodies and analysed by one- or two-dimensional gel analyses. *a*, *b* and *c*, One-dimensional SDS-PAGE analysis. Immunoprecipitations were performed on CHAPS solubilized R9, R2 or Ci1 T_S lymphocytes using mAb P3 (lanes 1 and 4), mAb βF1 (lanes 2 and 5), anti-CD3 mAb UCHT1 (lanes 3 and 6). Lanes 1–3 were analysed under reducing conditions (R), while lanes 4–6 were aliquots of the same immunoprecipitates analysed under nonreducing conditions (N). Immunoprecipitations from denatured (1% SDS) cell lysates were performed with control normal rabbit serum (NRS) (lane 7), rabbit anti-$C_{\gamma a}$ sera (lane 8), rabbit anti-$V_{\gamma b}$ (lane 9) and rabbit anti-$C_{\gamma c}$ (lane 10) all carried out under reducing conditions. *d*, *e*, Immunoprecipitates were performed on TX-100 solubilized J301 or J303 T_S lymphocytes using mAb P3 (lanes 1 and 3) or mAb βF1 (lanes 2 and 4) and analyses were carried out under R or N conditions. Immunoprecipitates from denatured (1% SDS) cell lysates were performed with control normal rabbit serum (lane 5) and with rabbit anti-$C_{\gamma a}$ (lane 6) under N conditions. *f*, *g*, Two-dimensional gel analyses of T_S T-cell receptors. Immunoprecipitates from ^{125}I-labelled CHAPS solubilized R9 (*f*) or R2 (*g*) lymphocytes were performed using anti-CD3 mAb UCHT1. The precipitates were separated by charge in the first dimension (NEPHGE) under R conditions followed by size separation (SDS-PAGE) in the second dimension, under R conditions.

Methods. One-dimensional SDS-PAGE. Viable lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation and 1×10^7 cells were radioiodinated by the lactoperoxidase technique as previously described[43]. Labelled cells were solubilized in 5 ml of TBS (10 mM Tris *p*H 8, 140 mM NaCl) containing 2 mM phenylmethylsulphonyl fluoride (PMSF) and 8 mM iodoacetamide (IAA), with 0.3% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulphonate (CHAPS) (*a*, *b*, *c*, *f* and *g* which preserve the TCR-CD3 association)[44] or with 1% Triton X-100 which dissociates the CD3–TCR complex (*d*, *e*). Immunoprecipitation was carried out using fixed *Staphylococcus aureus* Cowan I (SACI) as previously described[32], and the immune complexes were washed five times in TBS containing 0.1% Triton X-100 (TX-100). Rat anti-mouse κ chain-specific mAb 187.1 (5 μg) was added as a second antibody to provide protein A binding of IgG_1 mAb βF1, UCHT1 and P3[45]. Reduced samples were boiled in 2 mM dithiothreitol (DTT) and all samples incubated for 10 min at 23 °C in 10 mM IAA before analysis by SDS-PAGE. Immunoprecipitations using anti-C_γ sera were performed on ^{125}I-labelled T_S cells that were solubilized in 1% TX-100, dialysed to remove IAA, and then denatured in 1% SDS containing 3 mM DTT by boiling for 3 min. After partial renaturation by the addition of 4 volumes of 1.5% TX-100 in TBS containing 30 mM IAA, anti-C_γ sera or NRS were added, followed by SACI, and the immunoprecipitates were washed in TBS containing 0.5% TX-100, 0.5% deoxycholate, 0.05% SDS before analysis by SDS-PAGE. Anti-$C_{\gamma a}$ sera has been previously reported[24], anti-$V_{\gamma b}$ was generated against a 17 amino-acid synthetic peptide (Arg-Thr-Lys-Ser-Val-Thr-Arg-Gln-Thr-Gly-Ser-Ser-Ala-Glu-Ile-Thr-Cys) and anti-$C_{\gamma c}$ sera was generated against a 12 amino-acid synthetic peptide (Val-Pro-Glu-Lys-Ser-Leu-Asp-Lys-Glu-His-Arg-Cys), a gift from T Cell Sciences, Inc. (Cambridge, MA). For two-dimensional gel analysis, ^{125}I-labelled T_S cells were immunoprecipitated with UCHT1 as in *a*. NEPHGE (charge separation) was then carried out using *p*H 3.5–10 ampholines (LKB, Sweden), followed by 10.5% SDS-PAGE gels for size separation as previously described[26]. All gels were exposed to Kodak XAR film for autoradiography.

leprosy lesions following cytofluorographic cell sorting for $CD8^+$ cells as described previously[15,16,21]. To avoid the possiblity that mitogens might bind to the CD3–TCR complex and selectively expand cells with a certain type of receptor or otherwise bias the results, all clones were initially established by the method of limiting dilution cloning with propagation in the presence of interleukin-2 (IL-2) alone. The phenotypes and antigen-induced suppressor activity of these clones are summarized in Table 1. All the leprosy-derived lymphocyte clones were $CD3^+8^+4^-$ and $WT31^+$. These clones exhibited lepromin-induced suppression of the concanavalin A (Con A) response of peripheral blood mononuclear cells from normal donors as previously described[16]. Moreover, in five of five clones tested, the suppressor activity observed appeared to be MHC-restricted (Table 1 and ref. 16). On the basis of preliminary cytotoxicity data using lepromin-treated, MHC-compatible target cells that included human B cell lines, ori-sarcoma virus 40 transformed macrophage lines and 6-day primary cultured monocytes, we could find no evidence that the mechanism of supressor activity of these clones involved cytotoxicity of antigen presenting cells (data not shown). Similar findings were obtained by De Vries *et al.*[27].

In light of the known linkage between the cell-surface expression of CD3 and TCR αβ[28–32], or CD3 and TCR γδ[24] subunits, we undertook an investigation of CD3-associated TCR proteins on our T_S clones. T_S clones R9, R2 and Ci1 were surface iodinated, detergent solubilized (under conditions that preserve the CD3–TCR association), and labelled membrane proteins were immunoprecipitated using the anti-CD3 monoclonal antibody, UCHT1. In addition to the CD3 subunits (M_r 20,000–27,000 (20–27 K)), CD3-associated polypeptides were detected on all T_S clones (Fig. 1). When examined under reducing conditions, R9 and R2 cells expressed two CD3-associated species of 40–50K, while only one species was detected on Ci1 cells (Fig. 1*a*, *b*, *c*, lane 3 for each clone). When aliquots of these same immunoprecipitates were compared under nonreducing conditions, all of the CD3-associated species were noted to be disulphide-linked (Fig. 1*a*, *b*, *c*, lane 6 for each clone). Thus, human T_S clones R9, R2 and Ci1 express 40–50 K disulphide-linked, CD3-associated polypeptides.

To determine whether these species represented the protein products of TCR α or β genes, reactivity with framework monoclonal antibodies specific for the TCR αβ complex was examined. The WT31 monoclonal antibody preferentially reacts with TCR $\alpha\beta^+$ lymphocytes in cytofluorographic analysis[23]. All of the T_S clones exhibited positive staining with this mAb, suggesting that TCR αβ proteins were expressed on their cell surfaces (Table 1). However, this monoclonal antibody is inefficient in immunoprecipitation studies[23] such that it is often not possible to isolate surface proteins directly using this reagent.

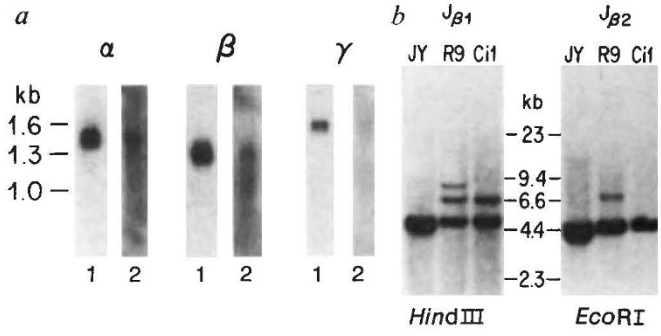

Fig. 2 Northern and Southern blot analysis of R9 RNA and DNA. *a*, Northern analaysis. Total RNA preparations from the T leukaemic cell line HPB-MLT (lane 1 for each probe) or from T_S clone R9 (lane 2 for each probe) were analysed on Northern blots using TCR α, TCR β and TCR γ cDNA probes. *b*, Southern analysis of TCR β gene rearrangements in R9 and Ci1 cells. Genomic DNA was isolated from a B lymphoblastoid cell line, JY (germline), and from Ts cells, R9 and Ci1. Genomic DNAs were digested with *Hin*dIII (Fig. 2*B*, $J_{\beta 1}$ probe) or with *Eco*RI (Fig. 2*B*, $J_{\beta 2}$ probe) and hybridized with ^{32}P-labelled $J_{\beta 1}$ and $J_{\beta 2}$ probes as indicated. **Methods.** *a*, RNA preparation, agarose gel electrophoresis, transfer to nitrocellulose, hybridization with ^{32}P-labelled, nick translated probes, and autoradiography were as described previously[24]. Approximately 0.5–1.0 μg of RNA was loaded per lane, probes were labelled to similar specific activity, and identical times of autoradiographic exposures are presented. RNA sizes were determined based on previously published lengths for TCR β and TCR γ transcripts[35,46]. *b*, Genomic DNA was isolated as previously described[47], digested with restriction enzymes *Hin*dIII or *Eco*RI, size fractionated on 0.7% agarose gels and transferred to nitrocellulose filters for hybridization with ^{32}P-labelled $J_{\beta 1}$ or $J_{\beta 2}$ probes, respectively. The organization of the human TCR β gene has been reported[48]. Probes are restriction enzyme DNA fragments of previously isolated human genomic clones[49]. $J_{\beta 1}$ is a 2.6-kilobase (kb) *Hin*dIII–*Nsi*I fragment containing the $J_{\beta 1}$ gene segment cluster. Because of a *Hin*dIII site in the 5′ end of $J_{\beta 1.1}$, the most 5′ of the $J_{\beta 1}$ gene segments, rearrangements to this particular segment may not be detected on Southern blot analyses of *Hin*dIII-digested genomic DNA. $J_{\beta 2}$ is a 4.4-kb *Eco*RI–*Eco*RI fragment containing the $J_{\beta 2}$ gene segment cluster. This probe detects rearrangements to the $J_{\beta 2}$ gene segment cluster on Southern blot analyses of *Eco*RI-digested DNA. The $J_{\beta 1}$ and $J_{\beta 2}$ probes do not cross-hybridize. After hybridization, filters were washed in 2 × SSC and 0.1% SDS at 23 °C followed by 0.2 × SSC and 0.1% SDS at 68 °C before autoradiography with intensifying screens. Because of the varying contribution of DNA from irradiated B lymphoblastoid cells used as a feeder layer, the 4.0 kb *Hin*dIII fragment containing the germline $J_{\beta 1}$ gene segment and the 4.4-kb *Eco*RI fragment containing the germline $J_{\beta 2}$ gene segments are present even in lanes containing two rearranged bands. The two filters were first hybridized to a TCR β constant region (C_β) cDNA probe which failed unequivocally to reveal rearrangements. The filters were therefore stripped in 0.5 M NaOH before the hybridizations shown (above) with the genomic $J_{\beta 1}$ and $J_{\beta 2}$ probes.

Thus, an additional monoclonal antibody (mAb), $\beta F1$, which has framework reactivity against the TCR β subunit and is capable of immunoprecipitating the disulphide-linked TCR $\alpha\beta$ complex from the surface of T lymphocytes[25] was used. This antibody immunoprecipitated polypeptides (40–50 K reduced; 80–90 K nonreduced) which appeared identical to those co-immunoprecipitated using anti-CD3 mAb (Fig. 1*a*, *b*, *c* lanes 2, 5 for each clone). Similar results were obtained using mAb βF1 for immunoprecipitation from radiolabelled Triton-X 100 solubilized J301 and J303 T_S cells. Under these conditions, CD3 and TCR do not remain associated such that the TCR α and β are isolated without CD3 (Fig. 1*d*, *e*). A second CD3-associated subunit could not be visualized by one-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) on Ci1 or J303 cells either because this species was poorly radiolabelled with ^{125}I or because both species were of similar SDS-PAGE mobility; and

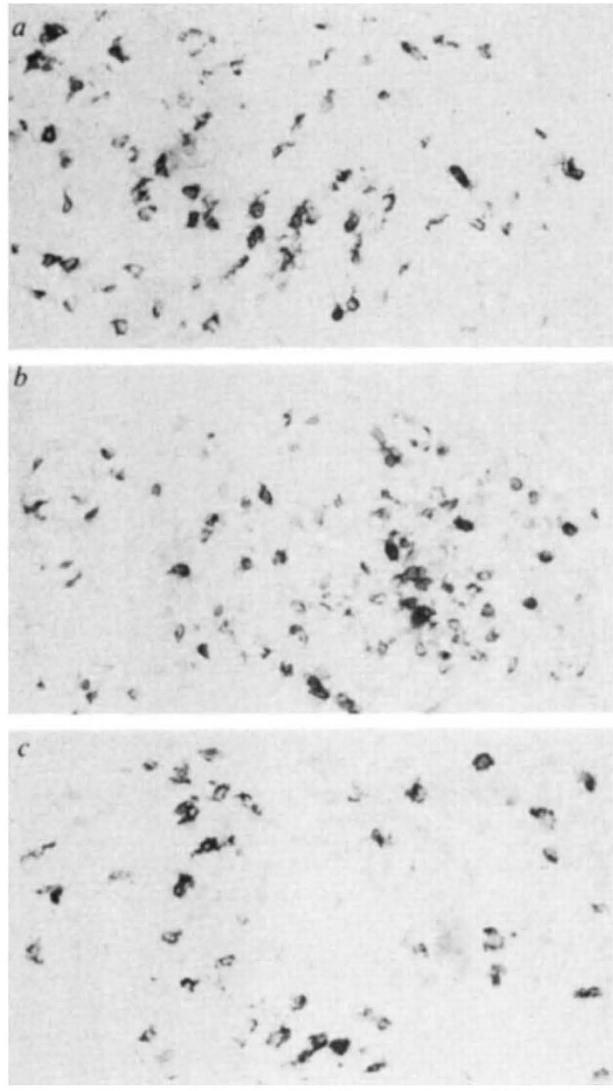

Fig. 3 Cells expressing CD3 and TCR β chains in lesions of lepromatous leprosy. In the serial frozen sections of a skin biopsy specimen from lepromatous leprosy patient R (from whom 2 of the 3 clones were derived, namely R9 and R2), approximately 40% of the infiltrating cells stained positively for CD3 (*a*, dark black rings are positive cells), 40% for TCR β chains (*b*) and 25% for CD8 (*c*) (×130). Double staining of $CD8^+$ cells in this lesion showed that >80% were $CD3^+$ and TCR β^+ (data not shown). **Methods.** Frozen sections of skin biopsy specimens from 14 lepromatous leprosy patients were stained by indirect immunoperoxidase and counterstained with haematoxylin[40] with a panel of monoclonal antibodies CD3 (Leu4, Becton Dickinson), CD8 (Leu2a) and TCR β chains (βF1) as described. Double staining of $CD8^+$ cells with CD3 and βF1 was performed on five lepromatous leprosy biopsy specimens using a double labelling technique. The first antibody was visualized with immunoperoxidase and aminoethylcarbazole to give a red reaction product and the second antibody was visualized with glucose oxidase showing a blue reaction product. The percentage of double staining cells could then be counted over several oil immersion fields.

two species were visualized under nonreducing conditions from R2 cells. When the CD3-associated species expressed on T_S clones R9 and R2 were examined by two-dimensional gel electrophoresis (nonequilibrium *p*H gradient electrophoresis (NEPHGE) followed by SDS-PAGE) under reducing conditions, clone-specific acidic 45–50 K species and basic 40–45 K species were observed (Fig. 1*f*, *g*). Thus, R2 and R9 displayed clonally unique, βF1 reactive, heterodimeric CD3-associated subunits characteristic of TCR $\alpha\beta$ complexes.

Table 1 Characterization of T_S clones

Clone	Phenotype (per cent) CD3	CD4	CD8	WT31	Suppression (per cent) MHC compat.	incompat.
R2	99	0	99	99	28	NT
R9	99	2	96	99	50	NT
Ci1	99	3	98	99	55	NT
J31	91	0	99	89	66	−8
J301	100	1	96	99	30	−13
J303	93	0	97	93	44	−32
J107	94	7	89	98	25	−10
J109	94	3	99	100	31	−3

Skin biopsy specimens were obtained from three lepromatous leprosy patients, R, Ci and J, and $CD8^+$ clones were established directly from lesion-derived cells by limiting dilution in the presence of irradiated feeder cells and IL-2 as previously described[15,16]. Clones R2 and R9 were maintained with irradiated feeder cells or alternately with IL-2 alone (Electronucleonics Inc.) or IL-2 plus phytohaemagglutin (PHA, Leucoagglutinin, Pharmacia) plus an autologous EBV transformed B-cell line[41]. Clone Ci1 and all J clones were maintained with irradiated feeder cells; or alternatively with IL-2 alone; or IL-2 plus conditioned medium containing T-suppressor cell growth factor[42]. Phenotypes were determined by staining clones with FITC-conjugated CD3, CD4 and CD8 (Coulter Inc.) or WT31 followed by FITC conjugated goat anti-mouse IgG and analysing with a FACS IV (Becton Dickinson). Suppressor activity was determined by measuring lepromin-induced suppression of the Con A response of normal peripheral blood mononuclear cells. Responding cells were MHC matched (compat.) for at least one class I and one class II antigen for assays of J clones, or were matched for only one MHC class II antigen for assays of R and Ci clones, or were fully mismatched (incompat.) or not tested (NT), in the presence of the 25% of the various clones[16].

To confirm the expression of TCR α and β on R9 cells, mRNA expression was determined by Northern blot analysis. Full length TCR α and both full length (1.3 kilobase pairs (kb)) and short (1.0 kb) TCR β mRNAs were detected in Northern blot analyses using ^{32}P-labelled TCR α and β cDNA probes, respectively (Fig. 2*a*). The presence of full length TCR α and β mRNA offers further evidence for expression of a complete TCR $\alpha\beta$ heterodimer.

Previous studies on murine T suppressor hybridomas indicated that TCR β genes were either germline or deleted[11–13]. Since TCR β mRNA and polypeptides were detected on the T_S clones examined here, it seemed that these lymphocytes would necessarily have rearranged their TCR β genes. To verify this, Southern blot analyses were performed on high molecular weight R9 and Ci1 DNA digested with *Hin*dIII and *Eco*RI, using ^{32}P-labelled $J_{\beta1}$ and $J_{\beta2}$ genomic probes, respectively[28]. Using these enzyme-probe combinations, $J_{\beta1}$ rearrangements were detected in both R9 and Ci1 DNA, while $J_{\beta2}$ rearrangements were detected only in R9 DNA. Thus, T_S clones were demonstrated to rearrange their TCR β genes (Fig. 2*b*). The clonality of R9 and Ci1 was supported by the demonstration that two or fewer rearranged DNA fragments from each sample hybridized to the $J_{\beta1}$ or $J_{\beta2}$ probes.

The expression of TCR γ mRNA in T_S cells is of particular interest, as γ mRNA is expressed at high levels in neonatal thymus[33,34] (when self-tolerance is presumably being induced) and in-frame, functional TCR γ mRNA has generally not been found in T_H or T_C clones[35–38]. TCR γ mRNA was undetectable in R9 cells (Fig. 2*a*), suggesting that a cell-surface TCR γ polypeptide would not be displayed by this T_S clone. To rule out the possible co-expression of a TCR γ polypeptide with TCR $\alpha\beta$ polypeptides on the T_S clones, TCR γ-specific antisera were used. Two such antisera have been shown previously to recognize the cell surface protein product of the rearranging TCR γ gene. (Anti-$C_{\gamma a}$, previously reported as anti-C_γ[24], reacts with all human TCR γ cells examined thus far, anti-$C_{\gamma c}$ reacts with a subset of TCR γ lymphocytes (M.B.B. unpublished data) and reactivity of anti-$V_{\gamma b}$ is under study.) Accordingly, aliquots of R9, R2, Ci1, J301 and J303 radiolabelled cell lysates were denatured, then partially renatured and subjected to immunoprecipitation with these antisera generated against synthetic peptides corresponding to three different 15–20 amino-acid residue segments of a deduced TCR γ amino-acid sequence[35,39]. None of these anti-TCR γ peptide reagents immunoprecipitated a TCR γ protein from the surfaces of these T_S cells (Fig. 1*a*, *b*, *c*, lanes 8–10 for each clone; and Fig. 1*d*, *e*, lane 6 for each clone).

Taken together: (1) the virtual absence of TCR γ mRNA; (2) the absence of reactivity with anti-C_γ sera; (3) the demonstration of TCR β gene rearrangements; (4) the expression of full length TCR α and β mRNA; (5) the successful isolation of CD3-co-immunoprecipitated, heterodimeric acidic and basic subunits; (6) the isolation of these same subunits using the TCR β chain reactive βF1 monoclonal antibody argue compellingly that the T_S cells examined here express TCR α and β, but not γ proteins.

It could be argued that the culture conditions used to propagate the leprosy skin lesion-derived T_S clones may have selected for a minor subset of $CD8^+$ cells. To determine whether the receptors found on these T_S clones are representative of the majority of cells infiltrating lepromatous lesions *in vivo*, we carried out immunoperoxidase staining and microscopic analysis of the lymphocytes present *in situ* in freshly isolated skin biopsies[40]. In specimens from 14 lepromatous leprosy patients, 43% of infiltrating cells were $CD3^+$, 23% were $CD8^+$ and 30% were $\beta F1^+$. As it was conceivable that a significant portion of the $CD8^+$ cells were $\beta F1^-$, we investigated the phenotype of the $CD8^+$ cells in specimens from five lepromatous leprosy patients with double immunoperoxidase-glucose oxidase staining. The majority (>80%) of primary $CD8^+$ cells in lepromatous lesions expressed the TCR $\alpha\beta$ complex detected by mAb βF1 (Fig. 3). The possibility that some of the remaining *in situ* $CD8^+$ cells might express the TCR $\gamma\delta$ protein complex has not been excluded.

In summary, while there may well be more than one cell type or immune mechanism that mediates suppression, the *in vitro* and *in vivo* studies presented here establish that TCR $\alpha\beta$ complexes are expressed on one subset of human, antigen-responsive suppressor T cells.

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Recovery of immunodeficient mice from a vaccinia virus/IL-2 recombinant infection

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Vaccinia virus recombinants that express cloned genes encoding antigens of unrelated infectious agents, such as hepatitis B virus[1] and human immunodeficiency virus (HIV)[2,3], provide a new approach to the development of live vaccines. Although there is evidence that genetically engineered vaccinia viruses have reduced pathogenicity[4] a major obstacle to their use as vaccines is that severe complications can occur after vaccination, especially in immunodeficient individuals[5,6]. We describe here a recombinant vaccinia virus expressing murine interleukin-2 (IL-2) and show that athymic nude mice infected with the recombinant virus resolve the virus infection rapidly whereas mice infected with control virus develop a progressive vaccinal disease. By incorporating the gene for IL-2 in live virus vaccines it may be possible to prevent the severe complications that arise in recipients with an impaired immune system.

IL-2 is an immunoregulatory, T-cell-derived molecule which is required for the clonal expansion of antigen-activated T cells[7]. A recombinant vaccinia virus expressing murine IL-2 was constructed to study the lymphokine's effect on virus growth and immunogenicity. As shown schematically in Fig. 2*a*, complementary DNA encoding murine IL-2 (ref. 8) was inserted into the *Hin*dIII F region of a vaccinia recombinant, VV-HA (ref. 9), which expresses the influenza haemagglutinin. The IL-2 recombinant virus, VV-HA-IL2, coexpressed haemagglutinin and IL-2 using the same vaccinia 7.5-kD promoter but from separate sites in the viral genome. Because the herpes simplex virus (HSV) thymidine kinase (TK) gene was used as a selectable marker for virus construction, a control virus VV-HA-TK, expressing HSV TK but not IL-2 was constructed. A significant amount of biologically active IL-2 was detected in supernatants from human 143B cells infected with VV-HA-IL2 within 4 h and reached maximum activity around 12 h after infection (Fig. 2*b*).

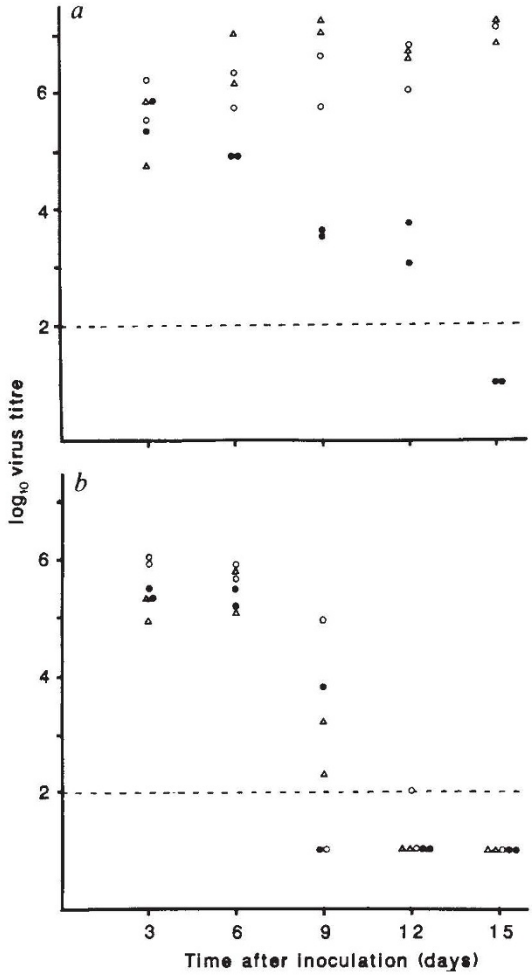

Fig. 1 Growth of vaccinia virus recombinants in the foot pads of athymic Swiss outbred nude mice (*a*) and euthymic CBA/H mice (*b*). VV-HA (△), VV-HA-TK (○) or VV-HA-IL2 (●) (2×10^7 PFU of each in 20 μl of saline) were injected subcutaneously into hind foot pads which were assayed for infectious virus on 143B cells on the indicated days. Points represent the titres of infectious virus present in individual mice.

Athymic nude mice (4–8 weeks old) were inoculated into the right hind footpad with VV-HA-IL2 or control virus (VV-HA-TK). VV-HA-IL2 induced a mild swelling in the foot which resolved after several days; in contrast VV-HA-TK produced a severe necrotic lesion that remained unresolved for 30 days. After this time, high titres of virus (6×10^5 to 1.5×10^7 plaque-forming units (PFU)) were recovered from the feet of the VV-HA-TK inoculated mice but not from mice given VV-HA-IL2. This suggested that the IL-2 produced by the recombinant virus enabled immunodeficient mice to control the virus infection. The kinetics of viral clearance from the feet of CBA/H mice were not significantly different for VV-HA-TK and VV-HA-IL2 (Fig. 1*b*). In nude mice, however, although titres of both VV-HA-TK and VV-HA-IL2 were high at day 3, indicating comparable rates of replication, VV-HA-IL2 was cleared by day 15, when no virus was detected in the feet. Titres of VV-HA-TK still remained high at day 15 (Fig. 1*a*). Furthermore, when nude

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