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T-cell receptor Vβ gene usage by lymphocytes infiltrating human renal allografts

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Abstract. T cell lines have been derived from human kidney allograft biopsies using mitogenic stimulation. Southern blotting using a T-cell receptor (TCR) Cβ probe revealed an oligoclonal pattern of rearranged bands in all 12 samples analysed. In some cases, differences in band patterns were noted between independent cultures from the same biopsy. Most T-cell clones derived from 2 biopsies showed different patterns of rearranged bands. The polymerase chain reaction (PCR) was used to study TCR Vβ gene usage in allograft-derived T-cell cultures. This was more sensitive and more informative than Southern blotting and revealed that most TCR Vβ genes were expressed in T cells from biopsies showing cellular rejection. The potential usefulness of this technique to quantify TCR V gene usage in allospecific T-cell populations is discussed.

Key words: Renal transplantation – T-cell receptor – Lymphocytes

The human T-cell repertoire for antigen is generated by somatic rearrangement of the T-cell receptor (TCR), variable diversity, and adjoining gene segments [1]. There are over 50 distinct TCR Vβ gene segments which can be assigned to around 20 subgroups on the basis of sequence homology and a similar diversity of TCR Vα segments which together contribute to the high degree of combinatorial diversity of the TCR αβ heterodimer [2]. In the mouse, T cells expressing certain Vβ genes predominantly recognise particular alloantigens [3] and are consequently deleted during thymic ontogeny in strains bearing that particular alloantigen [4, 5]. However, in man it is not clear whether T cells expressing particular Vβ genes are predominantly reactive with distinct alloantigenic determinants. If this is the case, a more specific form of immunosuppression might be possible in transplant patients using reagents against TCR gene products which are predominantly reactive towards the relevant mismatched HLA antigen(s).

Studies of small numbers of alloreactive T-cell clones have revealed a bias in TCR Vβ gene usage [6–8], but it is not known whether this is a general phenomenon and whether individual alloantigens preferentially stimulate selected T-cell subsets. Previous studies of rejecting human renal transplants have shown that infiltrating T cells stimulated either with donor alloantigen [9] or with interleukin 2 (IL-2) [10] have an oligoclonal pattern of TCR β gene rearrangements. We have used mitogenically stimulated T cells from renal allograft biopsies to estimate the degree of oligoclonality of the total cellular infiltrate. These studies have been extended by using the polymerase chain reaction (PCR) to identify TCR Vβ gene expression in these T-cell cultures. This has revealed that although T cells from rejecting allografts show oligoclonal patterns of TCR β gene rearrangement using Southern blotting, expression of most TCR Vβ genes can be detected in these infiltrates using the PCR.

Materials and methods

T-cell culture. Needle biopsies ca. 10 × 1 mm in diameter were taken from kidneys of transplant patients with deteriorating renal function. Biopsies were gently disaggregated in a small volume of phosphate buffered saline (PBS) using opposing 21 G needles and filtered through fine gauze. Cells were washed with PBS and resuspended in 3 ml RPMI 1640 plus 10% heat-inactivated fetal calf serum + antibiotics (RPMI-CS). They were then plated in 100-μl aliquots into 96-well, round-bottomed plates containing feeder mixture comprising RPMI-CS plus 2×10^5 allogeneic peripheral blood mononuclear cells/ml and 10^5 B lymphoblastoid cells/ml (both given 5000 rads γ-irradiation), 100 U/ml IL-2 (Biotest), 1 μg/ml phytohaemagglutinin (PHA; Wellcome), 1 μg/ml indomethacin (100 μl feeder mixture/well). This culture system results in clonal proliferation of almost 100% of peripheral blood αβ T cells [11]. Plates were examined after incubating for 10–16 days at 37 °C in an atmosphere of 5% CO_2, and in those samples in which almost all wells were positive for T-cell growth, cells from rows of 8 wells were pooled and

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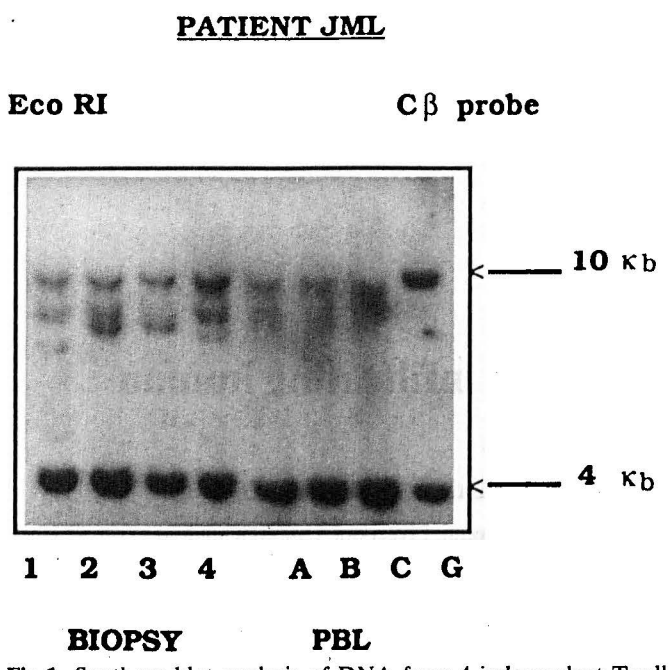

Fig. 1. Southern blot analysis of DNA from 4 independent T-cell populations *(1–4)* derived from the same kidney transplant biopsy from patient JML compared with PBL samples *(A–C)* from the same patient. *G*, germ-line DNA

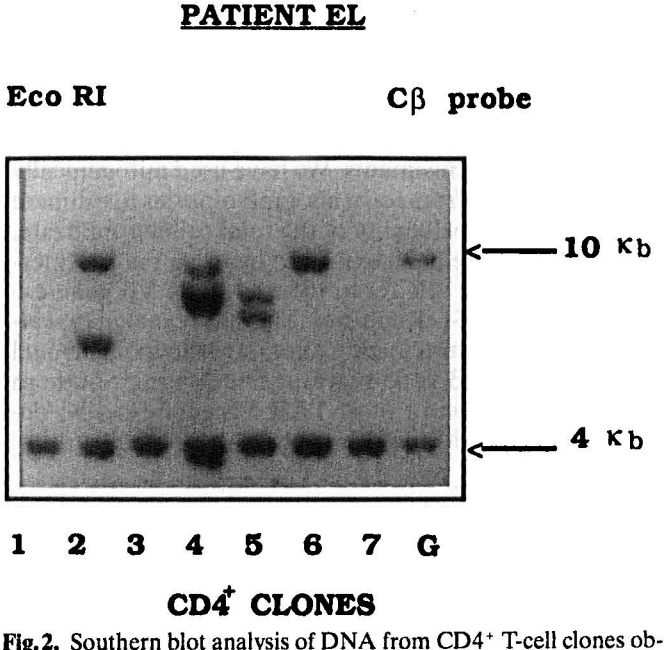

Fig. 2. Southern blot analysis of DNA from CD4+ T-cell clones obtained from a kidney transplant biopsy from patient EL

grown for a further 4–10 days in the same feeder mixture but without PHA. For most biopsies, 10^6–10^7 T cells were obtained from at least three independent aliquots of the same biopsy. In some experiments, T cells were cloned directly from the biopsy extract by plating out at limiting dilution as above and growing clones for 3–4 weeks prior to phenotypic analysis with monoclonal antibodies against CD3, CD4, and CD8 (Dako).

TCR β gene analysis. RNA was prepared from aliquots of $1–5 \times 10^5$ T cells from each biopsy using a micro-method [7]. The remainder of the cells were lysed in the presence of sodium dodecylsulphate (SDS) and proteinase K, and the DNA was extracted with phenol and chloroform and precipitated with ammonium acetate and ethanol [12]. The DNA was then dried and redissolved in TE buffer, pH 7.6. For Southern blotting, 10–15 μg aliquots were digested with *Eco*RI and electrophoresed in a 0.7% agarose gel. The DNA was transferred to a nylon membrane (Hybond N, Amersham) and hybridised with a ^{32}P-labelled TCR C probe (pB400, Dr. M.J. Owen, ICRF, London) [13]. For PCR analysis, the RNA was converted to cDNA with reverse transcriptase using a Cβ oligonucleotide primer [7]. The cDNA was divided into 20 aliquots and the PCR performed using unique primers for human TCR Vβ subgroups 1–20 together with a Cβ primer [7]. The PCR was carried out using 12 cycles of 1 min denaturation at 94 °C, 2 min annealing at 55 °C and 2 min extension at 72 °C followed by 20 similar cycles but with a 3 min extension time. The reaction was terminated with single cycles with extension times of 4.2 min and 5.2 min at 72 °C. The PCR products were then run on a 2% agarose gel and visualised in the presence of ethidium bromide. In some experiments, the PCR products were then blotted onto Hybond N and hybridised with an internal Cβ oligonucleotide end-labelled with γ-^{32}P-dATP. The relative intensities of bands obtained by ethidium bromide staining or autoradiography were estimated visually on a scale of 0–3 where 0 = band absent and 3 = strong band. Sensitivity and specificity controls were performed using cDNA from the T-cell line Jurkat, which expresses Vβ 8, and from normal peripheral blood lymphocytes (PBL). The former gave only a single band with the Vβ 8 primer and the latter gave bands with all 20 TCR Vβ primers as expected.

Results

Southern blot analysis

Of 60 biopsies studies, T-cell growth was noted in almost all wells in 26 (43%), most of which were diagnosed histologically as having cellular rejection. In all 12 cases in which multiple samples were analysed using Southern blotting, discrete rearranged bands were observed with DNA digested with *Eco*RI and hybridised with the Cβ probe (Fig. 1). In some cases, independent T-cell DNA samples from the same biopsy showed similar patterns of rearranged bands, but in others the rearranged bands sizes were clearly different in separate samples from the same biopsy. In contrast, PBL DNA from all samples showed a smear around the 7–10kb region in which no discrete bands were discernible (Fig. 1). T-cell clones were generated from biopsies from 2 patients with cellular rejection, and these comprised roughly equal numbers of CD4+ and CD8+ clones. When analysed using Southern blotting, most CD4+ clones had differently sized rearranged bands (Fig. 2), as did most CD8+ clones (data not shown).

Polymerase chain reaction experiments

Biopsy T-cell cDNA from 4 patients with cellular rejection and 1 with pyelonephritis was amplified by the PCR using TCR Vβ and Cβ primers. In the former 4 cases, the majority of the TCR Vβ genes were being expressed in the biopsy T-cell infiltrate. In 3 independent T-cell populations from the same biopsy (patient GC), a similar but not identical pattern of Vβ gene usage was found (Table 1). When biopsy T-cell populations were compared with PBL from the same patient, there was again a similar but not identical pattern of TCR Vβ gene usage (Table 2). A more

Table 1. Polymerase chain reaction (PCR) analysis of T-cell receptor (TCR) Vβ gene usage in 3 independent T-cell lines from the same biopsy from patient G. C. who was showing cellular rejection. The intensity of the bands stained with ethidium bromide was estimated according to the following scale: 3 = strong band, 2 = intermediate band, 1 = weak band, – = nothing visible

Biopsy	TCR Vβ Gene																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	3	1	2	2	3	–	–	2	1	–	1	2	2	2	–	–	–	1	–	–
2	2	2	3	1	1	1	2	2	1	–	–	1	3	2	–	–	–	2	–	–
3	2	2	3	2	2	–	2	3	1	–	1	1	2	2	1	1	2	–	–	1

Table 2. PCR analysis of TCR Vβ gene usage by biopsy T cells and PBL from patient B. G. who was showing cellular rejection. Band intensities were scored as in Table 1

	TCR Vβ Gene																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Biopsy	2	2	3	–	2	–	–	1	1	–	–	2	3	2	1	1	2	1	–	–
PBL	1	2	3	1	2	–	–	1	1	–	1	1	2	2	1	2	2	3	1	–

Table 3. PCR analysis of TCR Vβ gene usage in 2 biopsy T-cell lines from patient H. W. suffering from pyelonephritis as assessed by visualisation of an ethidium bromide-stained gel (EtBr) or by hybridisation with an internal Cβ probe (Probe). Band intensities were scored as in Table 1

	TCR Vβ Gene																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. EtBr	–	1	1	–	–	–	–	–	–	–	–	–	1	–	–	–	2	–	–	–
1. Probe	1	2	3	–	1	–	–	–	–	–	–	–	1	1	–	–	3	1	–	–
2. EtBr	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	2	–	–	1
2. Probe	2	1	2	–	1	–	–	1	–	–	–	–	3	2	–	–	3	1	–	2

sensitive method of detecting PCR products not visible by ethidium bromide staining was to hybridise then with an internal Cβ oligonucleotide probe (Table 3). This also showed that there was a considerably more restricted pattern of TCR Vβ gene usage in a biopsy from a patient subsequently diagnosed histologically as suffering from pyelonephritis rather than cellular rejection.

Discussion

We have used mitogenic stimulation to study TCR Vβ gene usage by the total T-cell population infiltrating renal allografts during a rejection episode. Although this does not take into account the specificity of T cells for donor alloantigen, in the absence of donor material this is a convenient way of analysing both donor-specific and nonspecific T cells within allograft biopsies. All 12 biopsies studied in detail had oligoclonal patterns of TCR β gene rearrangement as detected by Southern blotting. Statistically spealcing, each independent sample from a single biopsy contained the progeny of at least 30 T-cell clones, but in such samples only a few rearranged bands were visible, as has been reported previously [10]. However, results with the PCR showed that in all samples evidencing cellular rejection, the majority of the TCR Vβ subgroups was represented, indicating that Southern blotting is a relatively insensitive way of detecting T-cell polyclonality.

The PCR analysis of independent T-cell populations derived from the same biopsy showed similar patterns of TCR Vβ gene usage, indicating that the technique is reproducible. The similar pattern of TCR Vβ gene usage between the biopsy and peripheral T cells suggests either that there was a predominance of 'innocent bystanders' in the infiltrates studied or that T cells expressing a wide range of TCR Vβ genes can recognise a small number of alloantigens. The use of donor stimulator cells in in vitro culture experiments with allograft-derived T cells may reveal which of these is the case. A more accurate method of quantifying the PCR products would be needed to detect any relative increases or decreases in the proportions of T cells expressing different TCR Vβ genes following stimulation with alloantigen. Although the PCR products could readily be visualised in ethidium bromide-stained gels, the sensitivity of detection was increased by hybridisation with an internal Cβ oligonucleotide probe. This revealed bands which were not visible in the gel. The subjective method used for quantifying PCR products by estimating band intensities can undoubtedly be improved upon by using densitometry or by serially diluting PCR products [14] followed by probing with a Cβ probe, and future work will address this.

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