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throughput drug screening formats, growth in roller bottles and facilitated use in expression cloning experiments.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Ilana Stroke for the initial suggestion of using the MSR, and Dr. Stroke, Dr. Robert Swanson and Ms. Adriane Schilling for critical review of this manuscript.

REFERENCES

1. Ashkenas, J., M. Penman, E. Vasile, S. Acton, M. Freeman and M. Krieger. 1993. Structures and high and low affinity ligand binding properties of murine type I and type II macrophage scavenger receptors. *J. Lipid Res.* 34:983-1000.
2. Cheung, P.H., C.L. Webb, N. Aiyar, J. Disa, P.G. Lysko and G. Feuerstein (Eds.). 1997. Transient expression of CGRP receptor cDNA in genetically modified 293 cells; 1996 July 28–August 1; Montreal Canada. Peptide Receptors Symposium Proceedings; Abstract p. 1.12, p. 48.
3. Emi, M., H. Asaoka, A. Matsumoto, H. Itakura, Y. Kurihara, Y. Wada, H. Kanamori, Y. Yazaki et al. 1993. Structure, organization, and chromosomal mapping of the human macrophage scavenger receptor gene. *J. Biol. Chem.* 268:2120-2125.
4. Fraser, I., D. Hughes and S. Gordon. 1993. Divalent cation-independent macrophage adhesion inhibited by monoclonal antibody to murine scavenger receptor. *Nature* 364:343-346.
5. Horlick, R.A., K. Sperle, L.A. Breth, C.C. Reid, E.S. Shen, A.K. Robbins, G.M. Cooke and B.L. Largent. 1997. Rapid generation of stable cell lines expressing corticotropin releasing hormone receptor for drug discovery. *Prot. Expr. Purif.* 9:301-308.
6. Hughes, D.A., I.P. Fraser and S. Gordon. 1995. Murine macrophage scavenger receptor: in vivo expression and function as receptor for macrophage adhesion in lymphoid and non-lymphoid organs. *Eur. J. Immunol.* 25:466-473.
7. Hughes, D.A., I.P. Fraser and S. Gordon. 1994. Murine mPhi scavenger receptor: adhesion function and expression. *Immunol. Lett.* 43:7-14.
8. Matsumoto, A., M. Naito, H. Itakura, S. Ikemoto, H. Asaoka, I. Hayakawa, H. Kanamori, H. Aburatani et al. 1990. Human macrophage scavenger receptors: primary structure, expression, and localization in atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA* 87:9133-9137.
9. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. CSH Laboratory Press, Cold Spring Harbor, NY.

10. Shen, E.S., G.M. Cooke and R.A. Horlick. 1995. Improved expression cloning using reporter genes and EBV *ori*-containing vectors. *Gene* 156:235-239.

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Received 23 February 1998; accepted 17 April 1998.

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Detection and Characterization of $\alpha\beta$ -T-Cell Clonality by Denaturing Gradient Gel Electrophoresis (DGGE)

BioTechniques 25:244-250 (August 1998)

ABSTRACT

Accumulation of T cells carrying identical T-cell receptors (TCR) is associated with a number of immunological and non-immunological diseases. Therefore, it is of interest to be able to analyze complex T-cell populations for the presence of clonally expanded subpopulations. Here, we describe a simple method combining reverse transcription (RT)-PCR and denaturing gradient gel electrophoresis (DGGE) for rapid detection and characterization of T-cell clonality. The detection of clonally expanded T cells by DGGE relies on the fact that clonal transcripts have no junctional diversity and therefore resolve at a fixed position in the gel, which is determined by their melting properties. For polyclonal populations with a high degree of junctional diversity, the different DNA molecules will resolve at different positions in the gel and together will be revealed as a smear. For each of the TCR β -variable gene (BV)1–24 families, cloned transcripts were amplified and shown to resolve as distinct bands in the denaturing gradient gel, whereas the analysis of polyclonal T-cell populations resulted in a smear in the gel. The present method might prove useful to test for clonotypic T-cells in a variety of pathological and physiological conditions and for monitoring T-cell responses in diagnostic and therapeutic settings.

INTRODUCTION

The T-cell receptor (TCR) is a clonally distributed heterodimer, usually consisting of an α -chain and a β -chain. The T-cell repertoire is generated by rearrangement of gene segments encoding the TCR chains in the germ line consisting of a variable (V), joining (J) and constant (C) part, and for the β -chain, consisting of diversity (D) parts also. During this process, additional diversity

is introduced by the addition or deletion of nucleotides at the junction between the V/J and the V/D gene segments. This so-called "N-region diversity" provides a unique DNA sequence for each individual T cell, forming a clonotype (2). Several reports have shown that clonotypic T-cell responses are involved in the immune response associated with a number of both immunological (12,14,15) and non-immunological diseases (9,11). Therefore, it is of interest to be able to analyze mixed T-cell populations for the presence of clonal T cells. A number of different methods exist for the detection of T-cell clonality. The most widely used methods are based on reverse transcription polymerase chain reaction (RT-PCR) followed by single-strand conformation polymorphism (15), PCR heteroduplex analysis (13) or CDR3 size determination (8). However, all these methods require steps of either blotting and hybridization or re-amplification using end-labeled probes followed by computerized data analysis. We have developed a method based on RT-PCR and denaturing gradient gel electrophoresis (DGGE) to rapidly screen for the presence of clonal T cells. DGGE is a powerful method for the detection of small deletions, small insertions and point mutations, having a detection efficiency close to 100% (6). The method relies on the fact that the melting properties of a DNA molecule are highly dependent on the nucleotide composition. During electrophoresis, in a denaturing gradient gel, the DNA molecule will partially melt at a sequence-dependent concentration of denaturants, and the resulting partial separation of the DNA duplex will retard the molecule and thereby reveal a distinct band in the gel. This implies that even single base pair changes in a DNA sequence can be revealed in a denaturing gradient gel by a shift in the position at which the molecule is retarded (6). In a polyclonal T-cell population, all TCR DNA sequences will, in theory, differ from each other in their melting properties and therefore will be revealed as a smear in the denaturing gradient gel. In contrast, any population of clonally expanded T cells will be revealed as a distinct band that can be recovered for further analysis.

The DGGE-based method described

here provides a new tool for the detection of clonotypic T cells in mixed T-cell populations. The method is fast and excludes steps such as blotting, hybridization and re-amplification, as the presence of T-cell clonality is detected by a simple staining with ethidium bromide. The method is easy to handle and can be introduced even in more modestly equipped laboratories. The method will be of potential interest to researchers aiming at monitoring T-cell responses.

MATERIALS AND METHODS

In Vitro Culture of Peripheral Blood Lymphocytes

Samples of heparinized blood were obtained from healthy volunteers. Lymphocytes were separated by centrifugation on Lymphoprep™ (Nycomed, Oslo, Norway) and cultured in RPMI-1640 medium (Life Technologies, Gaithersburg, MD, USA) containing 10% fetal calf serum (FCS) for 4 days in the presence of 20 µg/mL phytohemagglutinin-P (PHA) (Sigma Chemical, St. Louis, MO, USA) for polyclonal stimulation or 25 µg/mL purified protein derivative (PPD) (Statens Seruminstitut, Copenhagen, Denmark) for specific stimulation. Cells were either stored as a pellet at -80°C or the RNA was isolated immediately.

Primer Sequences and Melting Maps

The primer sequences used for the amplification of TCR β-variable gene (BV) regions 1–24 are given in Table 1. Sequences of TCRBV regions were obtained from the GenBank® database using accession numbers given by Arden et al. (1). Using the PC/GENE™ program CLUSTAL (IntelliGenetics, Palo Alto, CA, USA), the sequences in each family were aligned, and primer sequences were found in regions of optimal consensus. The selection of optimal primers were aided by the computer program OLIGO™ Version 3.4 (Medprobe, Oslo, Norway) (10), aiming at a ΔG value of approximately -40.0 and a melting temperature (T_m) between 50° and 60°C. Melting maps for amplified PCR products were calculated using the MELT87 computer algorithm (5).

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Table 1. TCRB Oligonucleotide Primers for DGGE Analysis

BV	Sequence	Pos ^a	Size ^b	ΔG	T _m (°C)
BV1	5'-(GC ⁶)-ACTCTGAACTAAACCTGA-3'	-55	199	-41.3	51.2
BV2	5'-TCAACCATGCAAGCCTGACC-3'	-74	212	-39.3	55.7
BV3	5'-CGCTTCTCCCTGATTCTGGAGTCC-3'	-54	192	-47.1	61.2
BV4	5'-TTCCCATCAGCCGCCCAAACCTA-3'	-81	219	-49.9	65.0
BV5	5'-CTGAGATGAATGTGAGCACCTTG-3'	-51	189	-39.8	53.9
	5'-CTGAGCTGAATGTGAACGCCTTG-3'	-51	189	-43.4	58.6
BV6	5'-AGATCCAGCGCACAGAGCG-3'	-41	179	-39.3	56.2
	5'-AGATCCAGCGCACASAGCA-3'	-41	179	-37.6	54.9
BV7	5'-GCCAAGTCGCTTCTCACCTG-3'	-90	228	-39.3	54.8
BV8	5'-TGAAGATCCAGCCCTCAGAACCC-3'	-43	181	-45.3	60.2
BV9	5'-CTTCACATCAATTCCCTGGAGA-3'	-45	183	-39.7	53.1
BV10	5'-CCACGGAGTCAGGGGACACA-3'	-32	170	-39.9	57.8
BV11	5' TGCCAGGCCCTCACATACCTCTCA-3'	-31	169	-47.4	63.5
BV12	5'-GAGAATTTCTCCTCACTCTGG-3'	-47	185	-39.2	51.2
	5'-GACCTCCCCCTCACTCTGG-3'	-44	182	-37.8	53.0
BV13	5'-CTCAGGCTGCTGTCGGCTG-3'	-45	183	-39.2	56.8
	5'-CTCAGGCTGGAGTTGGCTG-3'	-45	183	-37.5	53.1
BV14	5'-AGGGTACAAAGTCTCTCGAAAAG-3'	-84	222	-40.4	50.7
BV15	5'-CAGGCACAGGCTAAATTCTCC-3'	-65	203	-40.2	53.1
BV16	5'-(GC ¹¹)-GGCGAACTGGAGATTCTGGAGT-3'	-30	181	-42.0	55.4
BV17	5'-GAAGGGTACAGCGTCTCTCGG-3'	-87	225	-41.0	55.3
BV18	5'-TTTCTGCTGAATTTCCCAAAGAGG-3'	-81	219	-45.7	57.7
BV19	5'-TCTCAATGCCCCAAGAACGCAC-3'	-74	212	-44.5	60.8
BV20	5'-AGGTGCCCCAGAATCTCTCAG-3'	-95	233	-40.3	54.7
BV21	5'-(GC ⁸)-GCTCAAAGGAGTAGACTCCACTCTC-3'	-65	211	-42.5	53.9
BV22	5'-AGATCCGGTCCACAAAGCTG-3'	-35	173	-39.2	54.0
BV23	5'-ATTCTGAACTGAACATGAGCTCCT-3'	-53	191	-41.1	53.0
BV24	5'-ATCCAGGAGGCCGAACACTTC-3'	-77	215	-42.0	56.8
BCseq2	5'-ACAGCGACCTCGGGTGGGAA-3'	38		-43.4	61.2
BC5'	5'-TGAGCTGGTGGGTGAATGGG-3'	187	343	-40.5	57.9
BC3'	5'-GGTGGCCTTCCCTAGCAGG-3'	530		-40.7	55.9
BCseq2-GC	5'-CCCGCCGCCCGCGCTCGCCCGCGCGCCCTGCCCGCGCCCCCGCCCGACAGCGACCTCGGGTGGGAA-3'				

^aPosition +1 is defined as the first nucleotide after the sequence coding for the conserved amino acid sequence CASS in the proximal end of the variable domain.

^bApproximate sizes of the PCR products are calculated using an estimated mean length of the DJ region of 50 bp.

RT-PCR

RNA was extracted using the PURE-SCRIPT® Isolation Kit (Gentra Systems, Minneapolis, MN, USA). cDNA synthesis was carried out using 1–3 µg of total RNA as previously described (4). TCR cDNA was quantitated using primers specific for the constant part of the β chain (BC5' and BC3') as previously described (12). Using the same amount of TCR template in each amplification, cDNA was amplified using primers specific for TCRBV regions 1–24 together with the "GC-clamped" constant region primer BCseq2. Amplifications were carried out in a total volume of 25 µL as previously described (12).

DGGE

Gels for DGGE analysis contained 6% polyacrylamide and a gradient of urea and formamide from 20%–80%

and were run at 160 V for 4.5 h in 1× TAE buffer (0.04 M Tris-base, 0.04 M acetate, 0.001 M EDTA, pH 8.3) kept at a constant temperature of 58°C. After electrophoresis, the gel was stained with ethidium bromide and photographed under UV transillumination. To demonstrate that all products could be resolved in the gel, PCR products for TCR-BV1–24 were cloned using the TA Cloning® Kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Positive bacterial clones were PCR-amplified for 30 cycles with the specific BV primer together with the GC-clamped BC primer, and 10-µL aliquots were analyzed using DGGE.

Quantitation of Clonotypic Transcripts

To investigate the detection sensitivity of the method, PCR was carried out using a ³³P-labeled BCseq2-GC primer

amplifying peripheral blood lymphocytes (PBL) cDNA intermixed in 5 different ratios with a clonal TCR transcript (BV17). Samples were loaded twice onto a denaturing gradient gel; the second loading was performed 30 min before the running time expired, enabling quantitation of the amount of clonal transcript (first loading) and the total amount of TCR transcript (second loading). Quantitation was carried out using the ImageQuant™ software (Molecular Dynamics, Sunnyvale, CA, USA). Relative abundance of the clonotypic transcript was calculated as clonotypic signal/total signal × 100.

RESULTS

Primer Sequences

Primers were selected with a goal to match all members of the BV family in

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question and to have 5 mismatches to all other families. Furthermore, the amplified products were chosen to be within a limited size range (Table 1). Some BV families (BV5, BV6, BV12 and BV13) were amplified using two different BV primers to match all members of these BV families. Because the amplification of BV13 was inhibited by the presence of both BV13 primers in the same tube, amplification of the BV13 family was carried out in two different reaction tubes.

Melting Maps

Using the MELT87 computer algorithm (5), melting maps for each amplified TCRBV region were calculated for the theoretical ability to resolve under

standardized conditions. For most of the 24 BV regions, a suitable melting profile could be obtained by the attachment of a 50-bp GC-clamp to the 5' end of the constant region primer BCseq2. A short GC-clamp (8–16 bp) was added to the 5' end of some BV primers (BV1 and BV21) to modulate the melting profile (Table 1).

DGGE Analysis of Cloned TCRBV1–24

Cloned TCR transcripts were PCR-amplified and subjected to DGGE analysis to investigate whether it was possible to resolve all 25 products in the same gel using standardized conditions. For each TCRBV family, several individual clones were amplified, and a

minimum of 4 different clones were evaluated by DGGE. In all cases, it was possible to resolve the product as a distinct band in the gel (Figure 1). To investigate the ability of the method to distinguish between different clones carrying the same TCRBV region, 16 different TCRBV13 clones were amplified and run in the same gel (Figure 2). The results clearly indicate that the method is capable of distinguishing between clones that differ only in the junctional region of the receptor. The occasional presence of more than one band in single lanes was only observed in the analysis of cloned TCR transcripts.

Analysis of PHA- and PPD-Stimulated T Cells

To verify that polyclonal T-cell populations will reveal a smear in the gel whereas the presence of clonotypic T cells will reveal a distinct band in the gel, T cells were stimulated with PHA and PPD, respectively, and analyzed using DGGE. Before DGGE analysis, agarose gel electrophoresis was used to test that all 25 reactions had successfully amplified products of the correct size without spurious amplification products. The results showed that the RT-PCR/DGGE analysis of polyclonal T

Figure 1. Denaturing gradient gel analysis of clonal TCR transcripts covering BV families 1–24. PBL cDNA was amplified with primers specific for BV families 1–24, cloned into pCR[®] 2.1 vector (Invitrogen) and re-amplified. PCR products were loaded onto a 20%–80% denaturing gradient gel and run for 4.5 h at 160 V at a constant temperature of 58°C. DNA was stained with ethidium bromide and photographed under UV light. The presence of more than one band in some lanes was only observed in the analysis of cloned TCR transcripts.

Figure 2. DGGE analysis of 16 different clonal TCRBV13 transcripts. BV13 transcripts were amplified from PBL cDNA. Subsequently, PCR products were cloned into pCR 2.1 vector, re-amplified and loaded onto a 20%–80% denaturing gradient gel and run for 4.5 h at 160 V at a constant temperature of 58°C. DNA was stained with ethidium bromide and photographed under UV light. The presence of more than one band in some lanes was only observed in the analysis of cloned TCR transcripts.

Figure 3. DGGE analysis of T cells stimulated specifically (with PPD) or nonspecifically (with PHA). PBLs were stimulated for 4 days with PPD or PHA, respectively. Cells were harvested, and the RNA was isolated for subsequent synthesis of cDNA. Before analysis with RT-PCR/DGGE, the amount of TCR cDNA was quantitated, and the individual amplifications were carried out using equal amounts of TCR cDNA.

Figure 4. Sensitivity of the DGGE method. T-cell clone cDNA was mixed with cDNA from PHA-stimulated T cells to enable the quantitation of clonal and polyclonal components in the sample (ratio of T-cell clone/PHA: lane 1, 1/4; lane 2, 1/8; lane 3, 1/16; lane 4, 1/32; lane 5, 1/64). Upper panel shows the sample after 30 min DGGE (before sequence-dependent separation); lower panel shows an equal amount of the same sample after 4.5 h DGGE (after sequence-dependent separation) resolving only the clonal transcript as a distinct band in the gel. The clonal transcript in the first four lanes was visible under UV light, after staining with ethidium bromide.

cells results in a gel smear, whereas the analysis of PPD-stimulated T cells demonstrated the presence of several distinct bands in the gel. Figure 3 shows examples of the DGGE analysis of PHA- and PPD-stimulated T-cell populations.

Quantitation of Clonotypic Transcripts in a Heterogeneous Background

To investigate the detection level of DGGE, experiments were carried out to quantitate the fraction of a clonotypic transcript in a mixed T-cell population. This was achieved by mixing at different ratios of T-cell clone cDNA with PBL cDNA. Dual loading at different time points enabled the quantitation of the clonotypic BV17 transcript relative to the total amount of TCRBV17 (Figure 4). This analysis showed that it is possible to detect clonal transcripts constituting below 2.5% of the total amount of PCR product (Figure 4, lane 4). The clonal transcript in the first four lanes was visible under UV light after staining with ethidium bromide.

DISCUSSION

We have described a novel approach for the detection of $\alpha\beta$ -T-cell clonality. The method combines RT-PCR to selectively amplify 24 TCRBV families and DGGE to resolve clonotypic T-cell sequences. Calculation of melting profiles of randomly selected TCRBV sequences formed the basis for selection of BV primers that, together with a GC-clamped constant region primer, amplify all relevant sequences and render them suitable for DGGE analysis. The versatility of the assay was demonstrated by the absence of bands in the gel when polyclonal populations were analyzed and by the presence of distinct bands in the gel when cloned transcripts from each of the 24 different TCRBV families were analyzed. Several cloned BV products from each family were analyzed using DGGE, and all of these were resolved as distinct bands in the gel. Furthermore, the analysis of 16 different cloned transcripts derived from TCRBV13 indicated that the present method has the resolving power to

distinguish between most clonal transcripts derived from the same TCRBV family. Obviously, situations can occur in which two different transcripts retard too close to each other to be recovered from the gel or even be distinguished as separate bands using "broad range" DGGE parameters (3). Nevertheless, our analysis of cloned TCR transcripts indicates that it is a rare situation to

have two different sequences that cannot be clearly distinguished as separate bands. This also makes the present method ideal for analysis of in vitro-raised, T-cell clones carrying the same BV region. When the amplified products are run in the same gel, the specific individual band pattern will reveal whether or not the clones are identical.

Most analyses of T-cell clonality

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focus on the examination of pathological tissues for the presence of clonotypic T-cells. Therefore, an important aspect is related to the sensitivity of the method. Using DGGE, clonotypic transcripts that constitute only a minority of the T cells carrying the specific BV region will be revealed in the gel. Clonotypic transcripts constituting as low as 2.5% of the BV region in question can easily be visualized in the gel by ethidium bromide staining. Assuming that all regions are expressed at equal levels, a T-cell clone can be detected in a mixed population at a fraction of 0.1%.

Currently, all methods for the analysis of clonality in TCRBV regions involve either different steps of blotting, re-amplification, radioactive labeling or exposure to autoradiographs (7,9,13,

15). The DGGE-based method described here requires none of these steps, and visualization of the amplified DNA is accomplished by means of simple staining with ethidium bromide. Therefore, the method is simple and fast and includes steps that can easily be set up for routine analysis, even in more modestly equipped laboratories.

The described method might provide a powerful tool to monitor T-cell responses in connection with vaccination protocols, immunotherapeutic transfusion of T cells and other situations where a T-cell clone is present as a minority in an otherwise polyclonal T-cell population.

ACKNOWLEDGMENTS

This work was supported by grants from the Danish Cancer Society.

REFERENCES

1. Arden, B., S.P. Clark, D. Kabelitz and T.W. Mak. 1995. Human T-cell receptor variable gene segment families. *Immunogenetics* 42:455-500.
2. Davis, M.M. 1990. T cell receptor gene diversity and selection. *Annu. Rev. Biochem.* 59:475-496.
3. Guldberg, P. and F. Güttler. 1994. "Broad-range" DGGE for single-step mutation scanning of entire genes: application to human phenylalanine hydroxylase gene. *Nucleic Acids Res.* 22:880-881.
4. Guldberg, P., P. thor Straten, A. Birck, V. Ahrenkiel, A.F. Kirkin and J. Zeuthen. 1997. Disruption of the MMAC1 gene by deletion or mutation is a frequent event in malignant melanoma. *Cancer Res.* 57:3660-3663.
5. Lerman, L.S. and K. Silverstein. 1987. Computational simulation of DNA melting and its application to denaturing gradient gel electrophoresis. *Methods Enzymol.* 155:482-501.
6. Myers, R.M., T. Maniatis and L.S. Lerman. 1987. Detection and localization of single base changes by denaturing gradient gel electrophoresis. *Methods Enzymol.* 155:501-527.
7. Offermans, M.T., R.D. Sonneveld, E. Bakker, P.P. Deutz-Terlouw, B. de Geus and J. Rozing. 1997. Denaturing and non-denaturing gel electrophoresis as methods for the detection of junctional diversity in rearranged T-cell receptor sequences. *J. Immunol. Methods* 181:101-114.
8. Pannetier, C., M. Cochet, S. Darche, A. Casrouge, M. Zoller and P. Kourilsky. 1993. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments. *Proc. Natl. Acad. Sci. USA* 90:4319-4323.
9. Pannetier, C., J. Even and P. Kourilsky. 1995. T-cell repertoire diversity and clonal expansions in normal and clinical samples. *Immunol. Today* 16:176-181.
10. Rychlik, W. and R.E. Rhoads. 1989. A computer program for choosing optimal oligonucleotide primers for filter hybridization, sequencing and in vitro amplification. *Nucleic Acids Res.* 17:8543-8551.
11. Sensi, M., C. Farina, C. Maccalli, R. Lupetti, G. Nicolini, A. Anichini, G. Parmiani and D. Berd. 1997. Clonal expansion of T lymphocytes in human melanoma metastases after treatment with a hapten-modified autologous tumor vaccine. *J. Clin. Invest.* 99:710-717.
12. thor Straten, P., E. Ralfkiaer, J. Hendriks, T. Seremet, G.L. Vejlsgaard and J. Zeuthen. 1998. T-cell receptor variable region genes in cutaneous T-cell lymphomas. *Br. J. Dermatol.* 138:3-12.
13. Wack, A., D. Montagna, P. Dellabona and G. Casorati. 1996. An improved PCR-heteroduplex method permits high-sensitivity detection of clonal expansions in complex T cell populations. *J. Immunol. Methods* 196:181-192.
14. Wilson, D.B., A.B. Golding, R.A. Smith, T. Dafashy, J. Nelson, L. Smith, D.J. Carlo, S.W. Brostoff and D.P. Gold. 1997. Results of a phase I clinical trial of a T-cell receptor peptide vaccine in patients with multiple sclerosis. I. Analysis of T-cell receptor utilization in CSF cell populations. *J. Neuroimmunol.* 76:15-28.
15. Yamamoto, K., H. Sakoda, T. Nakajima, T. Kato, M. Okubo, M. Dohi, Y. Mizushima, K. Ito and K. Nishioka. 1992. Accumulation of multiple T cell clonotypes in the synovial lesions of patients with rheumatoid arthritis revealed by a novel clonality analysis. *Int. Immunol.* 4:1219-1223.

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Received 2 February 1998; accepted 20 April 1998.

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