These results indicate that an autologous red cell agglutination test can be used as a sensitive and specific test for the presence of circulating antibodies. The procedure is flexible and could be tailored to detect a variety of antibodies for which there was an appropriate antigen for conjugation to the red blood cell antibody. In using the autologous red cell agglutination test for antibodies to HIV-1 it will be essential to use a conjugated antigen with a very low false negative rate. This may require the use of a mixture of peptide epitopes or soluble recombinant protein antigens. It is also possible that the test may be automated, just as automation has been achieved for the gelatin bead agglutination test (*2*) and many turbidimetric or nephelometric assays (*11*). Preliminary results indicate that the conjugated antibody is stable to freeze-drying and can be air-dried onto plastic plates. For field testing, a positive control mAb to the synthetic peptide antigen would be included as a means of quality control for the reagent.

At present the major advantages of the autologous red cell agglutination test are its speed and simplicity. For these reasons it is likely to have significant applications as a "front line" test. In this role it will be an important adjunct to conventional test procedures and may also be useful for assessing vaccination programs. It is hoped that the autologous agglutination test will contribute to the protection of health care workers and aid in the control of the spread of AIDS.

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8. Synthetic peptides were synthesized by the Merrifield procedure [R. S. Hodges and R. B. Merrifield, *Anal. Biochem.* **65**, 241 (1975)] with the aid of an Applied Biosystems Model 430 synthesizer using double coupling cycles supplied by the manufacturer. The *N-t*-butyloxycarbonyl amino acid derivatives were obtained from the Protein Research Foundation (Osaka, Japan). Side chain protection was the same as supplied by Applied Biosystems with the exception of arginine for which the ω-NO_2 derivative was used. Chain assembly was monitored with ninhydrin [V. Sarin *et al.*, *Anal. Biochem.* **117**, 147 (1981)]. The assembled peptides were simultaneously cleaved and deprotected by using anhydrous HF containing 10% anisol (v/v) [J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis* (Freeman, San Francisco, 1966), pp. 44 and 66]. The crude peptide was precipitated with diethylether and washed with ethyl acetate before being extracted with 60% acetonitrile in 0.1% trifluoroacetic acid (v/v). Synthetic peptides were purified by preparative reversed phase chromatography (Amicon C_{18} resin, 250 Å pore size 25 × 400 mm) with a gradient of 1000 ml, 0 to 60% acetonitrile in 0.1% trifluoroacetic acid. The synthetic peptide was approximately 95% pure as judged by analytical reversed-phase high-performance liquid chromatography and by quantitative amino acid analysis after acid hydrolysis.
9. Purified monoclonal antibody (10 mg) was treated with SPDP (110 μg) in phosphate-buffered saline (PBS), *p*H 7, for 60 min. Untreated SPDP was removed by chromatography on Sephadex-G25 using the same buffer. The derivatized antibody contained approximately 6 mol of propyldithiopyridine per mole of antibody as indicated by reduction in the presence of dithiothreitol. The synthetic peptide (3 mg) was reduced in buffer containing 0.1*M* tris-HCl, 1 m*M* EDTA, 4*M* guanidine hydrochloride, 160 m*M* 2-mercaptoethanol, *p*H 8, for 90 min at room temperature. The reduced peptide was recovered by batch chromatography on a Sep-pak (Waters Associates) and rotary evaporation from 60% acetonitrile in 0.1% trifluoroacetic acid. The reduced peptide was dissolved in 0.6 ml of 4*M* guanidine hydrochloride and immediately added to 6 mg of the derivatized antibody in 3.0 ml of 0.1*M* PBS, *p*H 7, containing 100 m*M* NaCl. After overnight incubation the degree of peptide substitution was measured spectrophotometrically at 343 nm. The conjugated antibody was separated from unreacted peptide by chromatography on Superose-12 using a Pharmacia FPLC system equilibrated with PBS. The fractions across the protein peak were assayed by the red cell agglutination assay, bulked, and concentrated in dialysis tubing with powdered Ficoll. Complete separation of the peptide-mAb conjugate from unreacted peptide and aggregated conjugate was essential. This procedure is reproducible, and nine independent peptide-mAb conjugates were prepared and found to be active in agglutinating seropositive patients' red blood cells. Active conjugate was also prepared by using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester as the cross-linking reagent.
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Selection of Variable-Joining Region Combinations in the α Chain of the T Cell Receptor

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Most T lymphocytes express an antigen-specific receptor composed of two subunits, α and β, each of which can exhibit structural variability. A complex selection process operates on T cells during development in the thymus such that cells expressing only particular αβ-receptors migrate to the periphery. The α-chain repertoire was dissected at different stages of the selection process by using the polymerase chain reaction (PCR) technique to amplify only those transcripts of a particular variable region gene (V_{58}). Sequences from these V_{58} cDNAs reveal the predominant expression of four joining (J) segments by T cells in the adult thymus, suggesting that molecular or cellular processes select particular $V_\alpha J_\alpha$ combinations during development. T cells expressing one of these $V_{58}J_\alpha$ chains appear to have been negatively selected at a later stage, since these transcripts were present in the spleen at approximately one-tenth the level in the thymus. Results also indicate that residues present at the $V_\alpha J_\alpha$ junction may be important in an early selection process.

T LYMPHOCYTES RECOGNIZE A FOREIGN antigen only when it is presented with a cell surface product of the major histocompatibility complex (MHC). The T cell receptor (TCR) responsible for this dual recognition is composed of two subunits, α and β, each generated by somatic rearrangement of multiple gene segments (*1, 2*). However, before a T lymphocyte becomes a functional antigen-specific cell in the peripheral lymphoid system, it undergoes selection in the thymus. The selection process, which operates on the αβ-heterodimer, guarantees that a T cell will react with a product of the MHC (a phenomenon resulting in MHC "restriction") and that it will not react with "self" antigens (a phenomenon resulting in self tolerance). The observation that >95% of cells in the thymus die (*3*) and never reach the periphery has long been suggested to be, at least in part, the result of this process.

Despite the progress that has been made in identifying the TCR, the structural basis of MHC restriction remains unknown. It has now been established that the αβ-heterodimer on mature T cells (helper and cytotoxic) is responsible for binding both antigen and MHC product on the presenting cell (*4*). In most cases, attempts to correlate

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the expression of particular α or β chains with restriction by specific class I or class II MHC products have met with little success (*5–7*).

Several studies, however, have demonstrated that expression of a particular V_β chain may be correlated with the MHC-restricting element. For example, T cells expressing the $V_{\beta 17a}$ chain are apparently eliminated in the thymus of mice expressing the MHC class II product, IE (*8*). In addition, a bias in V_β expression was observed among both cytochrome c–specific (*9*) and myoglobin-specific (*10*) class II–restricted T cells. Analysis of α chains from helper T cells specific for cytochrome c showed that there was also frequent use of a particular V_α gene and even a particular $V_\alpha J_\alpha$ combination (*6, 9, 11*). Since the T cells examined in these studies were all cytochrome c–specific and class II–restricted, it was unclear whether the limited α-chain repertoire reflected the response to the antigen or the restricting element or both.

In order to understand the selection process that leads to MHC restriction it would be advantageous to examine T cells before specific antigen stimulation. This is complicated by the tremendous diversity that can be generated in the αβ-heterodimer. The complexity of the α-chain locus in particular (>100 V genes, >50 J gene segments) (*12–14*) and the lack of suitable V_α-specific antibody reagents have made analyses of this chain difficult. We have begun to address these issues by using the polymerase chain reaction (PCR) (*15*) to amplify transcripts of a specific V_α gene in T cells from the thymus or the periphery. Amplified transcripts can then be analyzed by either hybridization with J_α gene probes or by sequencing. We show in this report that there is preferential expression of $V_\alpha J_\alpha$ region combinations and that the frequency of one $V_\alpha J_\alpha$ combination found in the spleen is approximately one-tenth that found in the thymus. Furthermore, N-region diversification is extensive with some $V_\alpha J_\alpha$ combinations and absent with others. The results imply that the J_α region, and the $V_\alpha J_\alpha$ junction in particular, of the TCR may play a critical role during the selection of cells in the thymus.

The extent of J_α segment diversity associated with a particular V_α gene was analyzed by using a primer that is specific for V_{58}, the V_α gene expressed by cytotoxic T lymphocyte (CTL) clone 2C [V_{58} is called pHDS58 in (*2*)] together with a C_α-specific primer for amplification of $V_{58}C_\alpha$ transcripts (Fig. 1). As judged from DNA blot data, the V_{58} gene family contains four to six members (*13*). Four cDNA sequences [pHDS58 (*2*), TA26 (*14*), 8/10-2 (*7*), and C9 (*7*)] that probably represent members of this family have >95% nucleotide sequence identity. The amplification primer (V_{58B}) differs from pHDS58 by 2 bp; this was done in order to generate a Bam HI site for cloning. It is otherwise identical to the sequences of pHDS58, TA26, and 8/10-2, and differs from C9 by 1 bp. An 18-base internal probe (V_{58}) has complete identity with pHDS58 and C9, five base mismatches with TA26, and two base mismatches with 8/10-2. PCR conditions were initially optimized with RNA from CTL 2C. The cDNA was synthesized with the C_α primer, and the PCR was performed in the presence of excess C_α and V_{58B} primers through 30 to 50 cycles.

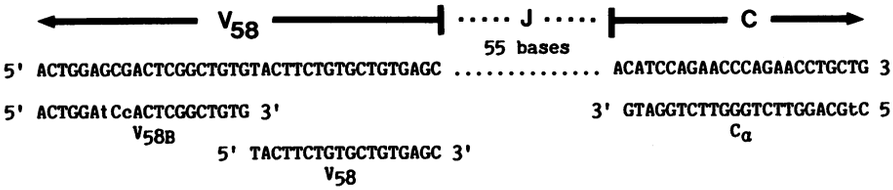

Fig. 1. Oligonucleotides used in the amplification and analysis of α-chain transcripts. Partial sequence of the gene encoding the α chain from the alloreactive, BALB.B CTL 2C is shown (pHDS58) (*2*). A constant region oligomer (C_α) was used as a primer for cDNA synthesis. The C_α oligomer and a variable region oligomer (V_{58B}) were used for amplification in the PCR reaction. The V_{58B} primer contained two base changes (lower case) in order to generate a Bam HI site (GGATCC), and the Cα primer contained a single base change in order to generate a Pst I site (CTGCAG) for cloning of amplified transcripts. An internal oligomer (V_{58}) was used for analysis of amplified transcripts.

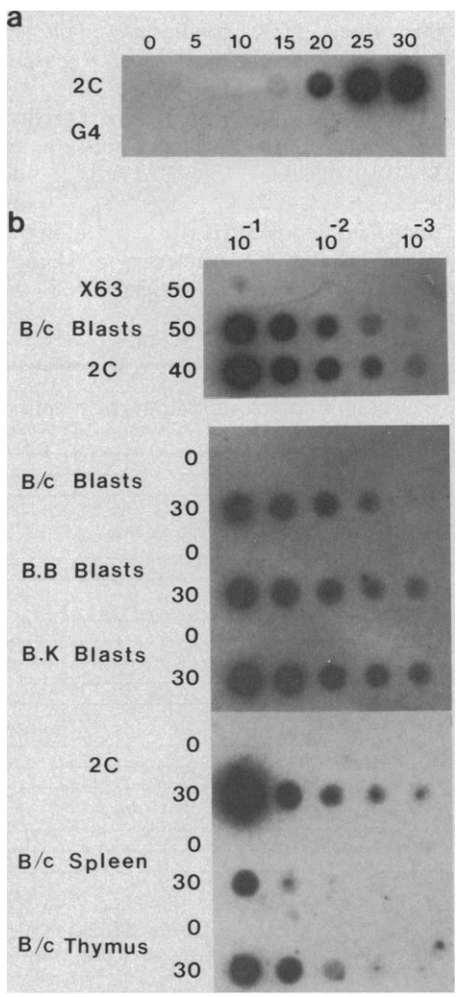

Fig. 2. Dot blot analysis of cDNA amplified by the PCR with the ^{32}P-labeled V_{58} probe. (**a**) Analysis of RNA from the two CTL lines, 2C and G4, at various cycles of amplification by means of the ^{32}P-labeled V_{58} probe. RNA was prepared by the guanidinium isothiocyanate–CsCl method (*24*). Approximately 10 μg of RNA was incubated with 7.5 μ*M* C_α, 1 m*M* deoxynucleotides, 4 m*M* sodium pyrophosphate, 50 m*M* tris-HCl (*p*H 8.3), 10 m*M* $MgCl_2$, 12 units of RNasin, and 50 units of reverse transcriptase in a total volume of 20 μl. After 30 min at 43°C, half of the material was brought to 100 μl in 67 m*M* tris-HCl (*p*H 8.8), 6.7 m*M* $MgCl_2$, 16.6 m*M* ammonium sulfate, 10 m*M* mercaptoethanol, 6.7 μ*M* EDTA, 150 μ*M* deoxynucleotides, 10% dimethylsulfoxide, and 2 μ*M* V_{58B}. The sample was denatured for 5 min (only 1 min for additional cycles) at 92°C and transferred to 50°C for 1 min to allow primer annealing. Three units of *Thermus aquaticus* polymerase were added (*25*), and the sample was incubated for 2 min at 65°C. This cycle was repeated 30 times with the addition of fresh polymerase at cycles 10 and 20. Five microliters of sample were removed before the first cycle (cycle 0) and after every five cycles. These samples were treated with 100 μl of 0.1 m*M* NaOH plus 0.1 m*M* NaCl and applied to GeneScreen Plus membranes (Du Pont Biotechnology Systems) in a 96-well dot blot apparatus. Blots were prehybridized (1% bovine serum albumin, 7% SDS, 1.5 m*M* EDTA, 250 m*M* NaCl, and 0.5*M* $NaPO_4$, *p*H 7.2) at 53°C for approximately 2 hours and V_{58} end-labeled with ^{32}P was added in the same solution at 53°C overnight. Blots were washed in 2× standard saline citrate (SSC) and 0.2% SDS at 53°C and exposed to film for 1 to 2 days. (**b**) Analysis of various dilutions of amplified RNA from polyclonal T cell preparations using ^{32}P-labeled V_{58} probe. RNA was prepared (as described above) from the mouse myeloma X63.653, CTL 2C, and Con A blasts (after 2 days of culture) of spleen cells from BALB/c, BALB.B, and BALB.K mice, and unstimulated cells from BALB/c spleen and thymus. α-Chain cDNA was synthesized as above except that approximately 3 μg of polyadenylated RNA from the blast preparations was used. After amplification for the indicated number of cycles, one-tenth of the sample was used to make 3.2-fold dilutions (10^{-1} to 10^{-3}), and these were blotted and probed with ^{32}P-labeled V_{58} as described above.

Specificity of amplification is shown in Fig. 2. RNA from CTL 2C is amplified, as detected by the internal ^{32}P-labeled V_{58} probe. RNA from CTL G4 (a BALB.B T cell line that expresses α-chain transcript but not the V_{58} gene segment) (*2*) and X63.653 (a mouse myeloma that does not express α-chain transcript) is not amplified under the same conditions (Fig. 2, a and b, respectively). However, RNA isolated from five different polyclonal T cell preparations [BALB/c spleen; BALB/c thymus; and splenic concanavalin A (Con A) blasts of three MHC congenic strains: BALB/c, H-2^d; BALB.B, H-2^b; and BALB.K, H-2^k] was successfully amplified as shown with the ^{32}P-labeled V_{58} probe (Fig. 2b). These preparations contain a heterogeneous mixture of T cells and thus express a relatively low level of the V_{58} transcript (no positives in 200,000 clones from a conventional λgt10 cDNA library of BALB/c splenic blasts) (*16*). From a comparison of standard concentrations of plasmid pHDS58 with amplified splenic RNA preparations we estimate that these PCR conditions result in >100,000-fold amplification of V_{58} transcripts (*17*).

To analyze V_{58}-J combinatorial diversity, we cloned amplified transcripts from BALB/c splenic blasts, BALB.B splenic blasts, and BALB/c thymus into pUC19 by using Pst I and Bam HI sites present on the 5′ ends of the PCR primers (Fig. 1). Approximately 1% of the ampicillin-resistant colonies were positive with a ^{32}P-labeled V_{58} probe. The sequences of 18 positive cDNA clones are shown in Fig. 3. It is clear that each of the sequenced α-chain cDNAs is a member of the V_{58} subfamily. Sixteen cDNAs were identical to this region of the α-chain V_{58} sequence of CTL 2C (the two exceptions had single base changes).

Of the 18 V_{58} sequences, 4 had rearranged to the J_{58} segment, 3 had rearranged to the J_1 segment, 5 had rearranged to the J_2 segment, and 3 had rearranged to the J_3 segment. Three V_{58} cDNAs contained J segments that were found only once (1/18). Sequences of the J_1, J_2, and J_3 segments have not, to our knowledge, been reported previously. Previous studies of α cDNAs isolated from thymocytes or T cell lines have shown that the J_α segment repertoire is significantly more diverse than the J segment repertoires of the known immunoglobulin or TCR gene families. In fact, random isolation of 19 α-chain cDNAs from thymus yielded 18 different J gene segments (*14*). We have shown that the proportion of VJ combinations in amplified cDNA reflects the actual level of these transcripts prior to amplification (*18, 19*). Thus it is clear that there are restrictions in the expression of particular V_{58}J combinations.

Four of the cDNAs contain a J gene segment (J_{58}) that is identical to that of the α chain from the BALB.B CTL 2C. Three of these cDNAs were derived from BALB/c splenic blasts and one was from BALB/c thymus. Since the germline V_{58} and J_{58} sequences are known (*13*), it was possible to determine unambiguously the extent of diversity at the VJ junction. There were no N-region additions or nucleotide deletions in any of the four cDNA sequences as previously shown for the 2C α-cDNA sequence (*13*).

The J_1 gene segment was present in one cDNA isolate from BALB/c splenic blasts, one from BALB.B splenic blasts, and one from BALB/c thymus. Thus, like J_{58}, J_1 appears to be expressed preferentially with the V_{58} gene segment although, unlike $V_{58}J_{58}$ chains, $V_{58}J_1$ chains apparently contain extensive N-region diversity [for example, up to 12 base positions that differ between BSP6 and CSP6 (Fig. 3)]. The J_2 gene segment was present in two cDNAs derived from BALB/c splenic blasts and in three from BALB.B splenic blasts. In contrast, the J_3 gene segment was found in three cDNAs isolated from thymus but not in any of the cDNAs derived from spleens. As with $V_{58}J_1$ chains, the $V_{58}J_2$ and $V_{58}J_3$ chains appear to exhibit extensive diversity at the VJ junction. Since the germline J_1, J_2, and J_3 sequences are not known we cannot exclude the possibility that the diversity is due to multiple J_1, J_2, or J_3 gene segments. However, we feel this is unlikely since most, if not all, J_α gene segments appear to be present as a single copy in the germline (*13, 20*).

The notion that T cells expressing particular $V_\alpha J_\alpha$ combinations may be selected in the

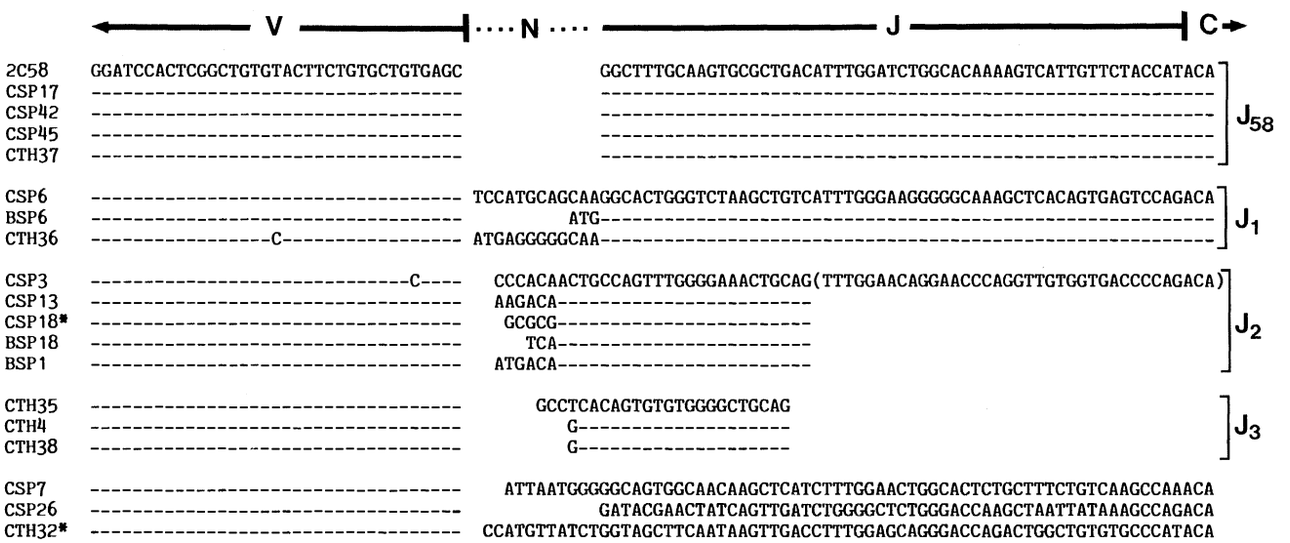

Fig. 3. Sequences of V_{58}^+ transcripts from BALB/c and BALB.B splenic blasts and BALB/c thymus. Amplified cDNA from BALB/c and BALB.B blasts and from BALB/c thymus (see Fig. 2) was digested with Pst I and Bam HI and cloned into pUC19. After transformation, ampicillin-resistant colonies were lifted onto GeneScreenPlus membranes and hybridized with ^{32}P-labeled V_{58} probe; positive colonies were isolated. Both strands of the inserts from plasmid preparations were sequenced by the dideoxy method. Several sequences that did not have a discernible J segment are not included. CSP isolates were derived from BALB/c splenic blasts, BSP isolates were derived from BALB.B splenic blasts, and CTH isolates were derived from BALB/c thymus. Sequences are aligned with the partial V sequence from CTL 2C (2C58) and a break is indicated at the V-J junction, with putative N regions present in some cases. Identical bases are indicated by a dashed line, and clones that use identical J segments (J_{58}, J_1, J_2, or J_3) are grouped. Since the J_2 and J_3 segments contain an internal Pst I site, the 3′ sequence could not be determined. However, we have sequenced a J segment (shown in parentheses for the CSP3 clone) from CTL line 2.1.1 that corresponds to the J_2 segment (*16*). Transcripts (CSP18 and CTH32) indicated by an asterisk have an out-of-frame sequence. These could be the result of aberrant transcripts or artifacts of either amplification or cloning.

thymus was examined further by blotting and hybridization. J_{58}, J_2, and J_3 oligomers labeled with ^{32}P were used to probe $V_{58}C_\alpha$ amplified transcripts from CTL 2C, BALB/c splenic blasts, BALB/c spleen, and BALB/c thymus (Fig. 4). Compared to the signal from V_{58} probe as a standard, we found that the relative abundances of J_{58}, J_2, and J_3 (as determined by densitometer tracing of autoradiograms) were consistent with the sequencing data discussed above. That is, the $V_{58}J_{58}$ and $V_{58}J_2$ chains are present in the spleen at approximately the same or slightly greater frequency than in the thymus. However, the $V_{58}J_3$ chain is expressed in the thymus at approximately ten times the level of that in the spleen or in the splenic blasts. This has been further shown by hybridization of V_{58}^+ colonies with the J_3 probe. The J_3 segment was present in 11 of 15 recombinants from thymus, none of 25 recombinants from spleen and none of 27 recombinants from splenic blasts.

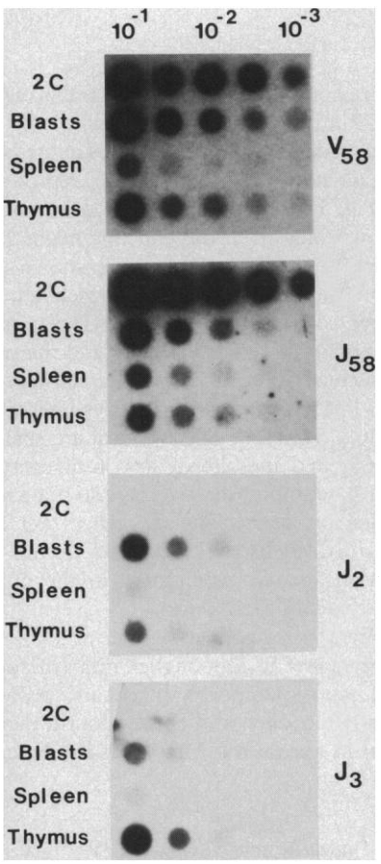

Fig. 4. Dot blot analysis of cDNA amplified by the PCR with ^{32}P-labeled V_{58} and J segment probes; cDNA from 2C, BALB/c splenic blasts, BALB/c spleen, and BALB/c thymus was amplified through 30 cycles and various dilutions (3.2-fold, 10^{-1} to 10^{-3}, as described in Fig. 2b) were blotted onto duplicate membranes and hybridized with probes corresponding to the J_2 segment (3′-GACGGTCAAACCCCTTTG-5′) or the J_3 segment (3′-GTGTCACACACCCCGACG-5′). After washing and exposure to film for about 1 day, blots were treated with 0.4*M* NaOH at 42°C to remove bound probe and rehybridized with a J_{58} probe (3′-TAGACCGTGTTTTCAGTA-5′). After the blots were washed and exposed to film, they were again stripped to remove probe and rehybridized to the V_{58} probe. Autoradiograms were scanned with a densitometer to determine intensities of J segment signal relative to V_{58} signal. Only those values within a range of 0.1 to 1.0 absorbance units were used to compute ratios (some values for data from the spleen were difficult to determine because of low signal). The V_{58}, J_{58}, J_2, and J_3 probes do not cross-hybridize with any of ten different α-gene cDNAs derived by conventional cloning from Con A blast libraries (*17*).

Previous studies of cytochrome c–specific T cells have also demonstrated preferential J_α segment use (*6, 9, 11*). However, the expression of a restricted repertoire of J_α gene segments with the V_{58} gene shown in this report must be due to selection mechanisms that occur prior to antigen stimulation. Several possible explanations may account for such restrictions. First, it could be due to preferential rearrangement events, analogous to the more frequent joining of J_H segments to the most proximal V_H regions during early B cell development (*21*). It is possible that the most 5′ J_α gene segments are the first to join to V_α segments. Although we do not know the germline positions of the J_1, J_2, and J_3 gene segments, the J_{58} gene segment is located approximately in the middle of the J_α cluster (*20*). Hence, J_α gene proximity does not fully explain frequent V_{58} to J_{58} joining, although other DNA elements could be involved in the preferential joining of V_α genes with some J_α gene segments. Another possible explanation for restricted α-chain expression is that only particular α chains can pair with β chains. These possibilities remain to be examined.

Preferential $V_\alpha J_\alpha$ expression could also be due to selection of T cells during development. The frequent appearance of $V_{58}J_{58}$, $V_{58}J_1$, $V_{58}J_2$, and $V_{58}J_3$ chains in the thymus may indicate that cells bearing these TCRs have survived due to reactivity with self-MHC determinants. We assume that this process requires the expression of appropriate β chains in association with these α chains (see below).

The observation that expression of the $V_{58}J_3$ α chain is reduced tenfold in the periphery suggests that T cells bearing this chain are prevented from migrating out of the thymus, perhaps because of reactivity with self components. This finding may be analogous to recent reports that T cells expressing $V_\beta 8.1$ (*22*) or $V_\beta 6$ (*23*) are deleted during development in the thymus of Mls^a-bearing mice.

Since BALB/c (H-2^d) and BALB.B (H-2^b) mice express identical (for example, $V_{58}J_{58}$) α chains in some of their mature T cells, other features of the TCR must distinguish H-2^d from H-2^b restricting elements in these cells. In addition, BALB/c, BALB.B, and BALB.K mice express approximately equivalent levels of $V_{58}J_{58}$ transcripts in the thymus and spleen (*17*). Thus, T cells expressing the $V_{58}J_{58}$ chain may be an example in which the β chain is the determining factor for distinguishing polymorphic MHC determinants as shown for other T cells (*8, 10*).

The extensive $V_\alpha J_\alpha$ junctional diversity present in some combinations ($V_{58}J_1$, $V_{58}J_2$, and $V_{58}J_3$) suggests that the N region may also play an important role in ligand (Ag/MHC) binding. Interestingly, each of the three $V_{58}J_1$ junctions contains a methionine residue (codon ATG) generated by nucleotide addition, and each of the three $V_{58}J_3$ junctions contains an alanine residue (codon GCC or GCA) generated by nucleotide addition. It is possible that specific amino acid side chains at this location within the α chain are critical for binding. Amino acid position 100 (a residue at the V-D junction) in the β chain from cytochrome c–specific T cells is relatively invariant, suggesting that it may be involved in binding either cytochrome c or the class II MHC product (*11*). Hence, it is likely that residues encoded by the N regions of both the α chain and the β chain are important contact residues. Since TCRs containing the α chains described in this report are presumably not reactive with the same nominal antigen, it is possible that MHC determinants may be the ligands for these interactions.

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26. We thank A. Horwitz and L. Schook for comments on the manuscript. Supported by NIH grant AI24635 (to D.M.K.) and the Searle Scholars Program/the Chicago Community Trust (to D.M.K.).

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Technical Comments

Triassic-Jurassic Extinctions

P. E. Olsen *et al.* (*1*) add an important new data point to the emerging pattern of Late Triassic and Early Jurassic extinctions and survivals among terrestrial vertebrates. The principal value of the new Nova Scotian quarries is their rich record of diversity within a tightly defined temporal interval at a point early in the Jurassic. Yet, as the authors note, no new presences or absences of taxa are recorded in this fauna that are not already known from other Early Jurassic localities. What further inferences are warranted?

The authors suggest two extreme hypotheses of the pace of Late Triassic extinction events (catastrophic versus gradual, on the order of "tens of millions" of years). Data already suffice (*2–4*) to place the extinctions of these groups within the Late Norian [5 million years (my) or less]. The new report brings the temporal range of these extinctions to less than 1 my—although only in the Newark Supergroup. This level of temporal and geographic resolution is far too coarse to permit a choice between "catastrophic" and more gradual ecological hypotheses, even if special explanation of the extinction pattern were warranted.

Olsen *et al.* base their hypotheses of rapid extinction in the latest Triassic on evidence stratigraphically below the new Nova Scotian sites. Latest Triassic correlation data come from a remarkable variety of sources, including pollen and spores, radiometric dates, bones and footprints of vertebrates, and remarkably fine microstratigraphic correlations of the Newark lake beds. Olsen *et al.* infer that the pattern of latest Triassic extinctions in the Newark Supergroup is representative of global change; it may be, but do the data show this? Of the 61 families and higher taxa of terrestrial vertebrates shown in their figure 2, only 23 (fewer than 40%) are known in the Newark. Of the 34 taxa that approach or cross the Triassic-Jurassic boundary, only 17 (50%) are present in the Newark. Moreover, all 17, as represented in the Newark, are (i) monotypic—represented by only one genus and species—suggesting the group was already on the wane; (ii) represented by scrappy material that cannot be identified below the high taxonomic level listed—thus providing no real measure of the pace of change of diversity; or both (i) and (ii). Four extinctions (three of which are obviously pseudoextinctions) occur in the next 2-my interval (*3*, figure 25.6, reproduced in Fig. 1).

The Newark's scrappy osteological record, even supplemented by its rich but temporally spotty footprint record, cannot support generalizations about the pace of extinction of latest Triassic taxa. During the last 2-my interval of the Triassic, only two taxa made their last appearances in the Newark (*1*, note 18; *3*, figure 25.6, reproduced in Fig. 1). These were the procolophonids, known only from the endemic genus *Hypsognathus*, and the phytosaurs, known only from indeterminate remains generally referred to the genus *Rutiodon*. The possible phytosaur footprint *Apatopus* and the pseudosuchian footprint *Brachychirotherium* are also last represented during this interval (*3*, figure 25.9). The affected taxa are sparsely represented and already low in diversity by that time; moreover, two taxa do not a mass extinction make. The 12 other taxa that breathed their last sometime during the Norian are not represented in the uppermost part of the Newark.

What seems equally significant is the first appearances of four higher taxa (theropod and sauropodomorph dinosaurs, protosuchian crocodiles, and trithelodontid therapsids) in the earliest Jurassic of the Newark.

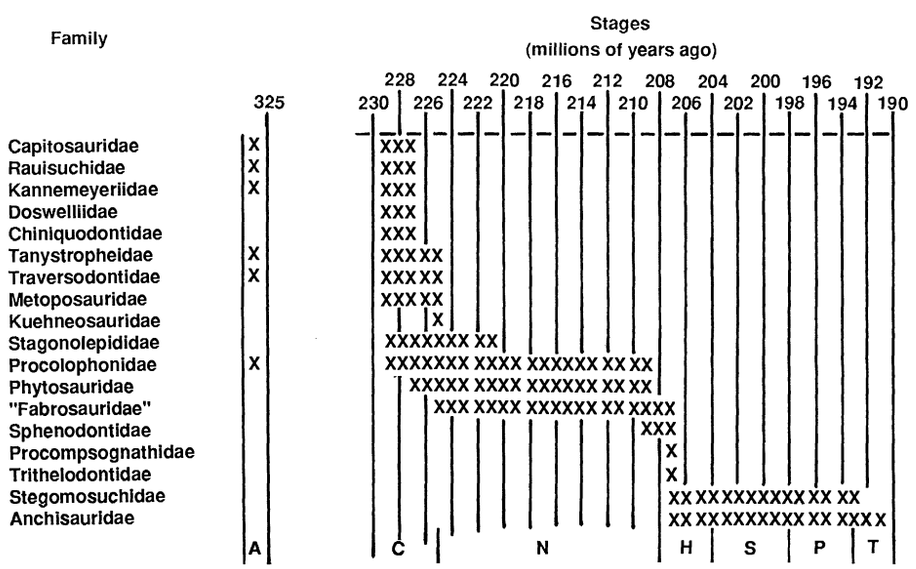

Fig. 1. Distributions of skeletal remains of tetrapods in the Newark Supergroup (from *3*). The patterns shown here by the 2-my scale contrast with those shown by the coarser scale of Triassic-Jurassic stages in figure 2 of Olsen *et al.* (*1*). The Triassic-Jurassic boundary is set at 208 my ago, between the Norian (N) and Hettangian (H) stages. Other abbreviations for stages are as follows: A, Anisian; C, Carnian; S. Sinemurian; P, Pliensbachian; T, Toarcian.