2B4.11 (*2*). Furthermore, we have found that antibodies directed against CD3, which are mitogenic for normal T cells (*20*), induce both IL-2 production and growth inhibition in a murine chemically induced T-cell lymphoma, EL-4, and a human T-cell leukemia, Jurkat (*21*). These results demonstrate that activation-induced growth inhibition is not a property unique to T-cell hybridomas and suggest that mitogenic antibodies to CD3, or perhaps other common and nonclonally distributed T-cell surface structures such as CD2 (*22*), may prove useful in the treatment of T-cell neoplasms of unknown antigen specificity.

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Structurally Divergent Human T Cell Receptor γ Proteins Encoded by Distinct Cγ Genes

MICHAEL S. KRANGEL, HAMID BAND, SHINGO HATA, JOANNE MCLEAN, MICHAEL B. BRENNER

The human T cell receptor (TCR) γ polypeptide occurs in structurally distinct forms on certain peripheral blood T lymphocytes. Complementary DNA clones representing the transcripts of functionally rearranged TCR γ genes in these cells have been analyzed. The expression of a disulfide-linked and a nondisulfide-linked form of TCR γ correlates with the use of the Cγ1 and Cγ2 constant-region gene segments, respectively. Variability in TCR γ polypeptide size and disulfide linkage is determined by the number of copies and the sequence of a repeated segment of the constant region. Thus, Cγ1 and Cγ2 are used to generate structurally distinct, yet functional, T3-associated receptor complexes on peripheral blood lymphocytes. Tryptic peptide mapping suggests that the T3-associated TCR γ and δ peptides in the nondisulfide-linked form are distinct.

THE T CELL RECEPTOR (TCR) γ gene, like those encoding the TCR α and β polypeptides, is composed of immunoglobulin-like gene segments, which are joined through somatic rearrangement during T cell differentiation (*1–3*). The human TCR γ locus consists of at least five functional variable (V) region, five joining (J) region, and two constant (C) region genes (*4–11*). Although the total number of functional V and J region genes is limited, significant diversity is introduced during the process of V–J joining (*3, 8, 12*). However, in the majority of functional T cell lines and tumors examined to date, the V–J joining process fails to maintain an open reading frame, and a functional TCR γ protein is not synthesized.

The use of antisera against TCR γ-specific peptides has led to the identification of T3-associated TCR γ on some peripheral blood and thymic T cells, as well as a leukemic T cell line (*13–17*). We have characterized two examples of peripheral blood TCR γ lymphocytes in detail (*13, 16*). The IDP2 cell line expresses a T3-associated TCR γ peptide of 55 kD (40 kD nonglycosylated) which immunoprecipitates along with a 40-kD peptide termed δ. The γ and δ polypeptides on the surface of IDP2 are not disulfide-linked. In contrast, PBL C1 cells express a T3-associated TCR γ peptide of 40 kD (31 kD nonglycosylated), which is disulfide-linked. Although a distinct partner chain has not yet been identified, indirect evidence suggests that this disulfide-linked TCR γ peptide is part of a heterodimer, and not a homodimer. Both IDP2 and PBL C1 appear to function as cytotoxic T lymphocytes (*16*).

The present study was conducted to elucidate the molecular bases for the structural differences among the TCR γ and δ peptides on IDP2 and PBL C1. In particular, we wished to investigate the dramatic differences in both the size and disulfide linkage of the TCR γ peptides on these cells and the structural relation between TCR γ and δ on IDP2.

Complementary DNA (cDNA) libraries were prepared from IDP2 and PBL C1 poly(A)$^+$ RNA in the vector λgt10 (*18*) and were screened by hybridization with ^{32}P-labeled human TCR γ cDNA clone pTγ-1 (*6*). Clones were selected for detailed analysis on the basis of both size and limited restriction enzyme mapping, which suggested that they represented the transcripts of rearranged TCR γ genes. Four PBL C1 clones and the one IDP2 clone so selected lacked Kpn I sites, suggesting rearrangement to Vγ9 (*7*). Nucleotide sequence analysis indicated that all PBL C1 clones arose from identical transcripts. The nucleotide sequence of the longest of these, PBLC1.15 (1.5 kb), is compared with that of IDP2.11 (1.4 kb) in Fig. 1. PBLC1.15 contains approximately 150 bp of 5′ noncoding region preceding a presumed initiator methionine codon and extends through a canonical poly(A) addition site at its 3′ end. IDP2.11 contains less 5′ sequence but also extends through a poly(A) addition site at its 3′ end.

The PBLC1.15 and IDP2.11 V regions are nearly identical to each other and to the coding region of a Vγ9 genomic sequence (*7*). Differences between PBLC1.15 and IDP2.11 at nucleotides 226, 230, and 234 are probably the result of reverse transcriptase errors in the region of the IDP2.11 hairpin loop. In addition, a difference at nucleotide 511 likely represents a reverse

M. S. Krangel, H. Band, S. Hata, J. McLean, Division of Tumor Virology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.
M. B. Brenner, Division of Tumor Virology, Dana-Farber Cancer Institute, and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

transcriptase error in PBLC1.15, since three other PBL C1 clones are identical to PBLC1.15 in all regions sequenced but agree with IDP2.11 and not PBLC1.15 at this position. Otherwise, these sequences differ from the published Vγ9 sequence at three nucleotides (171, 298, and 398) and in the assignment of the splice between the exon encoding the leader peptide and that encoding the main body of the V region. The splice occurs 18 bp upstream from the position predicted from the genomic sequence, and thus Vγ9 would be six amino acids longer than previously proposed. PBLC1.15 and IDP2.11 appear to use Jγ regions of identical nucleotide sequence (presumably corresponding to Jγ1.3 and Jγ2.3) (*7, 8, 11, 12*) and remain in frame across the V–J junction but differ by two nucleotides at the junction. Whereas IDP2.11 displays the genomic Jγ sequence at these positions, PBLC1.15 is distinct, presumably as a result of template-independent N-region diversity (*3, 12, 19*). These substitutions result in a single amino acid difference (Tyr versus Asn) at the V–J junction. This single difference throughout the entire V and J regions is notable, because PBL C1 and IDP2 display spontaneous cytolytic activity of differing specificity when tested on a panel of tumor targets (*16*). However, the relative importance of junctional diversity, the constant region used, or other polypeptides in modulating specificity cannot be assessed at present.

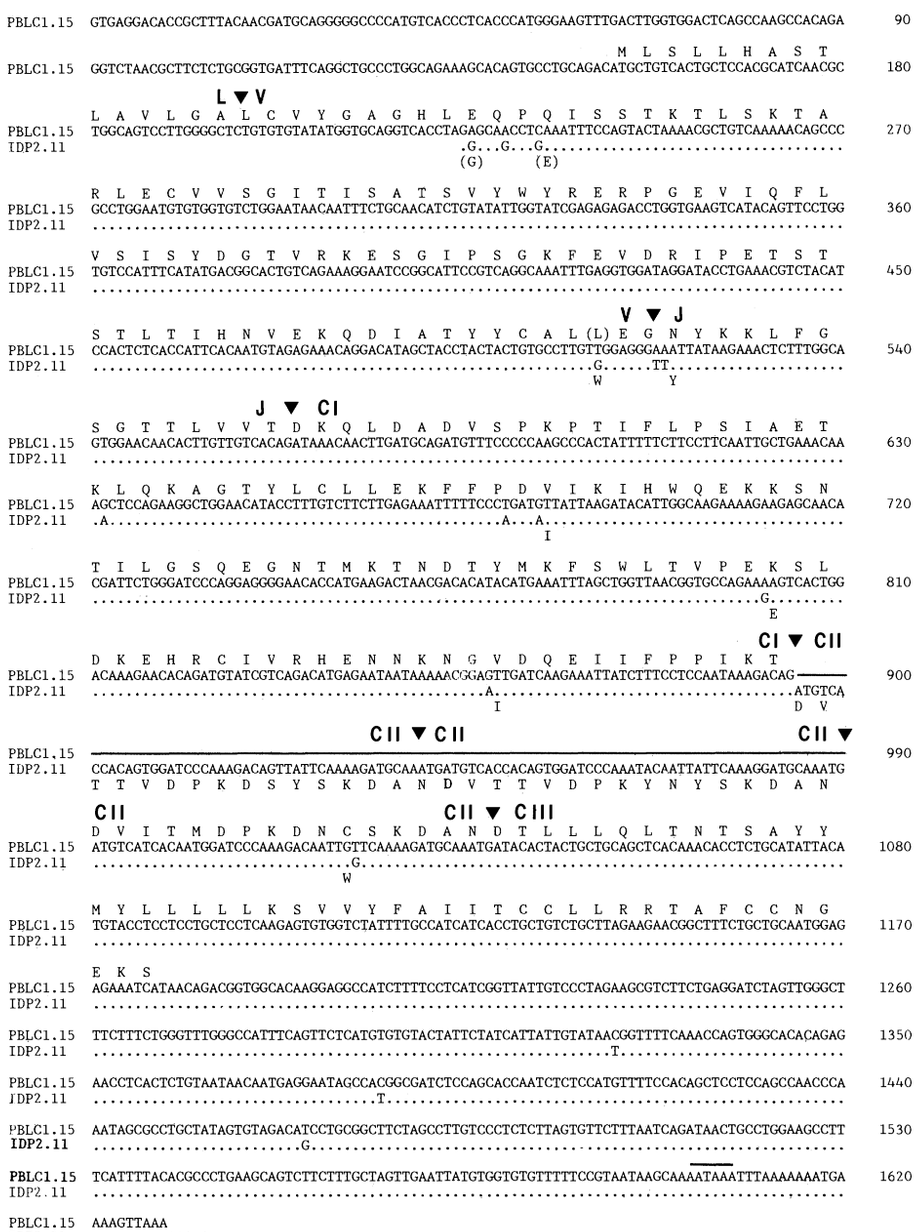

Fig. 1. Nucleotide sequences of PBL C1 and IDP2 TCR γ cDNA clones. Clones PBLC1.15 and IDP2.11 were sequenced in their entirety by the dideoxy chain termination method (*22*). The numbering scheme is for the aligned composite nucleotide sequence. Leader (L), variable (V), joining (J), and constant (CI, CII, CIII) region junctions are demarcated by comparison to the appropriate genomic sequences (*7, 9, 12*). IDP2.11 nucleotides identical to those of PBLC1.15 are denoted by the dotted line; differences are noted. The deduced amino acid sequence of PBLC1.15 is presented in the single letter code, and residues at which IDP2.11 differs are noted. The one-letter symbols for the amino acids are A, alanine; R, arginine; N, asparagine; D, aspartic acid; C, cysteine; Q, glutamine; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine; and X, unknown or "other." Amino acid assignments based on presumed reverse transcriptase errors are marked in parentheses. A large gap in the PBLC1.15 sequence relative to IDP2.11 is denoted by the solid line. The canonical polyadenylation signal is overlined.

The C regions of PBLC1.15 and IDP2.11 are clearly distinct. The PBLC1.15 sequence corresponds closely to a recently published Cγ1 sequence (*9*), and IDP2.11 to Cγ2 (*6, 9*). Notably, Cγ1 encodes a cysteine residue located on the amino-terminal side of the presumed membrane-spanning region, and this cysteine is absent in Cγ2 (*6, 9*) (Figs. 1 and 2). Since the TCR γ peptide is disulfide-linked on PBL C1 but not disulfide-linked on IDP2, this suggests strongly that this cysteine is responsible for interchain disulfide linkage. The region in Cγ1 around this cysteine is encoded in a small 48-bp exon (CII) (*9*). Strikingly, sequences homologous to this exon appear to be triplicated in IDP2.11. Copies of the repeat differ by one to five nucleotides, and all substitutions but one result in amino acid differences (Fig. 2). Copies 2 and 3 of this repeat were reported as distinct exons in the genomic sequence of Cγ2 (*9*). However, copies 1 and 3 have previously been observed in the out-of-frame HPB-MLT cDNA clone pTγ-1 (*6*). Whether this variability results from polymorphisms in the number of CII exons in Cγ2 genes or from alternative splicing remains to be determined. Aside from this, the PBLC1.15 C region sequence is identical to that of one allele of Cγ1 (*9*), but IDP2.11 differs from published Cγ2 sequences at two positions (1143 and 1368) (*6, 9*).

Except for the variable CII exon usage, the two constant regions are virtually identical. Because of the triplicated region, the IDP2.11 sequence predicts a polypeptide backbone 32 amino acids (3.8 kD) longer than that predicted by PBLC1.15. However, by SDS–polyacrylamide gel electrophoresis (SDS-PAGE), the nonglycosylated TCR γ peptides on PBL C1 and IDP2 differ by approximately 9 kD, and the glycosylated peptides differ by 15 kD (*16*). In order to investigate this apparent discrepancy, and as further proof that clones PBLC1.15 and

```
PBLC1.15  CII       AT GTC ATC ACA ATG GAT CCC AAA GAC AAT TGT TCA AAA GAT GCA AAT G
IDP2.11 CII copy 1  .. ... .C. ... G.. ... ... ... ... .G. .A. ... ... ... ... ... .
IDP2.11 CII copy 2  .. ... .C. ... G.. ... ... ... T.. ... .A. ... ..G ... ... ... .
IDP2.11 CII copy 3  .. ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... .

PBLC1.15 CII        ASP VAL ILE THR MET ASP PRO LYS ASP ASN CYS SER LYS ASP ALA ASN
IDP2.11 CII copy 1  ... ... THR ... VAL ... ... ... ... SER TYR ... ... ... ... ...
IDP2.11 CII copy 2  ... ... THR ... VAL ... ... ... TYR ... TYR ... ... ... ... ...
IDP2.11 CII copy 3  ... ... ... ... ... ... ... ... ... ... TRP ... ... ... ... ...
```

Fig. 2. CII repeat sequences. IDP2.11 CII repeats are aligned with the copy in PBLC1.15. Identities in the nucleotide and predicted amino acid sequences are marked by dotted lines; differences are noted. Potential sites for asparagine-linked glycosylation in this region are marked by underlining.

IDP2.11 encode TCR γ polypeptides with properties similar to those synthesized by the respective cell lines, we examined the polypeptides encoded by these clones after in vitro transcription and translation. Since IDP2.11 did not contain the entire coding region, it was repaired by ligating the 5′ portion of PBLC1.15 at the Nde I site within the V region. This was possible since the two clones use the same V region. After subcloning PBLC1.15 and IDP2.11 into pSP65 and transcribing them in vitro, in vitro translation products were analyzed by immunoprecipitation with an antiserum to a TCR γ–specific peptide (*13*), followed by SDS-PAGE (Fig. 3). PBLC1.15 and IDP2.11 directed the synthesis of TCR γ peptides of approximately 31 kD and 38.5 kD, respectively. These values agree well with those obtained by endoglycosidase-H treatment of immunoprecipitates from metabolically pulse-labeled cells (*16*). Thus, the large difference in nonglycosylated polypeptide size noted previously clearly results from usage of Cγ1 versus Cγ2. The anomalously large difference in SDS-PAGE mobility (31 kD versus 38.5 kD, but 34.7 kD versus 38.5 kD calculated from the deduced amino acid sequence) may result either from unusual SDS binding properties or a unique conformation of the triplicated portion of the polypeptide. It is also noteworthy that the Cγ1 sequence carries three or four potential sites of *N*-linked glycosylation (depending on the use of an asparagine residue next to the cysteine implicated in interchain disulfide bonding), whereas the Cγ2 sequence carries five. This corresponds well to the properties of the TCR γ peptides characterized on PBL C1 and IDP2. *N*-linked carbohydrate contributes approximately 9 kD to the former, but 15 kD to the latter.

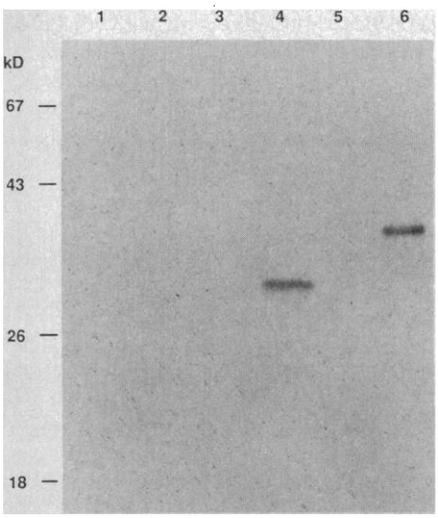

Fig. 3. In vitro transcription and translation analysis. IDP2.11 was repaired with the 5′ portion of PBLC1.15 by ligation at the Nde I site within the V region to generate IDP2.11r. PBLC1.15 and IDP2.11r were subcloned into pSP65, the plasmids were linearized with Hind III, and capped transcripts were synthesized with the use of SP6 polymerase (*23, 24*). In vitro translation in the presence of [^{35}S]methionine was performed in a rabbit reticulocyte lysate (*25*). Samples were denatured and immunoprecipitated with either normal rabbit serum or antiserum to TCR γ–specific peptide, as described (*16*), and immunoprecipitates were analyzed by 12% SDS-PAGE followed by fluorography (*26*). Translations were (lanes 1 and 2) no RNA, (lanes 3 and 4) PBLC1.15 transcripts, and (lanes 5 and 6) IDP2.11r transcripts. Immunoprecipitations were (lanes 1, 3, and 5) normal rabbit serum and (lanes 2, 4, and 6) antiserum to Cγ.

These results argue persuasively that the distinct properties of the TCR γ peptides on PBL C1 and IDP2 are the result of the use of Cγ1 versus Cγ2. Since both TCR γ peptides are associated with T3, since monoclonal antibody to T3 can induce an increase in cytoplasmic calcium and cytotoxicity for both cells, and since the cells display spontaneous cytotoxic activity against certain tumor targets (*13, 16, 20*), both TCR γ C region genes can be used to generate functional TCR γ–T3 complexes on peripheral blood lymphocytes. Precisely how the dramatic variability introduced into the region encoded by the CII exon affects receptor function remains to be determined. This region of the polypeptide may be an extended connector that links the CI exon–encoded immunoglobulin-like domain to the CIII exon–encoded membrane-spanning and cytoplasmic regions. The CII repeat region is predicted to carry much of the *N*-linked carbohydrate (Fig. 2), which may be important in protecting this segment of the polypeptide from proteolysis. Since this connector region appears to mediate interchain disulfide linkage of Cγ1, it may be of general importance in mediating interactions of TCR γ with a partner chain. Use of a distinct version of this region in Cγ2 may be coupled to the expression of and interaction with a distinct partner chain. In this light, it is notable that all but one nucleotide substitution between the different copies of the CII exon result in amino acid substitutions (Fig. 2), suggesting that their divergence has been under selective pressure. Whether functional Cγ2 products use other CII exon combinations [copies 1 and 3 in an HPB-MLT transcript, which is nevertheless out of frame at the V–J join (*6*); copies 2 and 3 in a genomic clone (*9*)] remains to be determined. Since a 40-kD nondisulfide-linked form of TCR γ has been detected on peripheral blood T cells (*16*) and on a thymic clone (*14*), this possibility remains open.

The identities of the T3-associated, putative TCR γ partner chains are still a mystery.

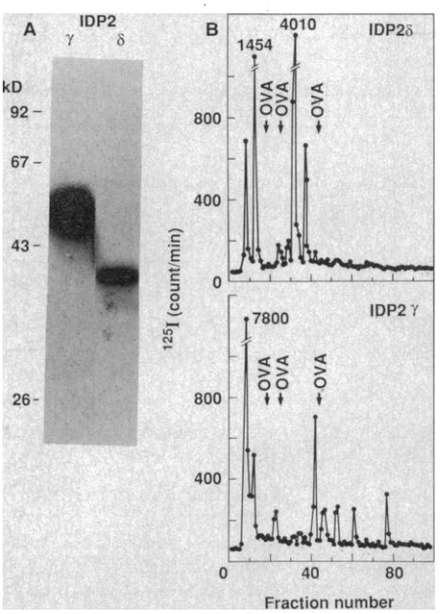

Fig. 4. Comparative tryptic peptide maps of IDP2 TCR γ and δ peptides. Preparative anti-T3 immunoprecipitates from surface-iodinated cells were resolved by SDS-PAGE, and TCR γ and δ bands were excised, eluted, and lyophilized. (**A**) Purified TCR γ and δ were examined by SDS-PAGE. (**B**) Alternatively, 15,000 count/min each were combined with cold carrier ovalbumin, reduced, alkylated, and trypsin-digested as described (*27*). Chromatography was performed on a Waters HPLC system with a Vydac C_{18} column and a linear gradient of 20% to 60% acetonitrile in 0.1% trifluoroacetic acid. The migration of carrier ovalbumin peptides was determined by monitoring absorbance at 235 nm and served as an internal control. The identical positions of three major ovalbumin peaks (OVA) demonstrate the two chromatograms to be directly comparable.

However the δ chain that we can visualize on surface-iodinated IDP2 is clearly distinct from the TCR γ peptide on these cells. It is unreactive with antisera to TCR γ–specific peptides (*13*). Further, tryptic peptide comparisons by reversed-phase high-performance liquid chromatography (HPLC) demonstrate the patterns of iodinated TCR γ and δ peptides to be quite different (Fig. 4). Only one or two peptides at or near the flowthrough appear to comigrate. Since the gel-purified TCR δ is known to be contaminated at low levels with underglycosylated TCR γ, the TCR δ peptide at fraction 8 is probably a TCR γ contaminant that is visualized because of its heavy labeling. The overall dissimilarity between these profiles suggests that IDP2 δ could not be related to IDP2 γ simply by proteolysis or other post-translational modifications. Thus the δ peptide appears to be a distinct component of the nondisulfide-linked TCR γδ-T3 complex. Whether this component is related to the partner chain in the disulfide-linked form and whether either of these molecules displays structural properties common to TCR α, β, and γ subunits will be subjects of intensive investigation.

Note added in proof: A structure similar to that described for IDP2 TCR γ has recently been reported for TCR γ from the PEER cell line by Littman *et al.* (*21*).

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A Mab to a Unique Cerebellar Neuron Generated by Immunosuppression and Rapid Immunization

SUSAN HOCKFIELD

The cerebellar cortex is perhaps the best characterized structure in the mammalian central nervous system. Although the major cerebellar cell classes are well known, a new class of cerebellar cortical neuron has now been identified with a monoclonal antibody (Mab) generated by a procedure for rapid immunization and selective immunosuppression of antibody responses. This procedure generates a high frequency of immunoglobulin G–class antibodies of desired specificity, and has allowed the generation of two antibodies that recognize subsets of cerebellar cortical neurons. One of these antibodies defines a previously unrecognized class of cerebellar neuron. The distribution and antigenic characteristics of this neuron suggest that it has a distinct role in cerebellar circuitry.

CURRENT VIEWS OF THE DERIVATION and maintenance of cell structure are that molecular differences must underlie the anatomical and physiological differences observed among neuronal classes in the mammalian central nervous system. Several recent studies suggest that the number of molecularly distinct cell classes in the central nervous system may be quite large (*1, 2*). To determine how molecular differences might give rise to or reflect neuronal heterogeneity, we and others have used hybridoma technology to immunologically dissect the biochemical constituents of the vertebrate (*3–8*) and invertebrate (*9–11*) central nervous system.

Although initially promising, the hybridoma approach has proven to have both biological and technical limitations so that few monoclonal antibodies to neuronal subsets have been generated. We have encountered three major obstacles. (i) It has been difficult to target the immune response to recognize antigens of interest; the vast majority of antibodies generated recognize a few, immunodominant species. (ii) Animals must be immunized over long periods of time to generate a hyperimmune response that will yield high-affinity, immunoglobulin G (IgG) antibodies. (iii) The large number of hybridoma colonies derived from the spleen in a successful experiment is difficult to screen immunohistochemically. For example, in our earlier experiments to obtain antibodies to molecules expressed by subsets of neurons, we immunized mice with a tissue that contained a high density of neuronal cell bodies. The spinal cord provided a convenient tissue source, because spinal gray matter contains neuronal cell bodies (intermixed with axons and glial cells) and can be dissected cleanly from the peripheral white matter, which is composed almost exclusively of axons and glial cells. After immunizations of mice with spinal cord gray matter, we screened antibodies immunocytochemically on spinal cord sections and found that, even after enrichment by dissection, only a small fraction of the antibodies generated recognized gray matter antigens, and the vast majority of antibodies recognized axons and glial cells (*3, 7*). Biochemical analyses showed that the axonal and glial antibodies recognized intermediate filaments (*5, 7*), immunodominant species in the central nervous system. Clearly then, physical separation of wanted (gray matter) from unwanted (white matter) antigens did not sufficiently attenuate the immunodominant response to axons and glia.

Here I describe techniques obviating these difficulties. These techniques have made it possible to generate antibodies that recognize restricted populations of neurons and, in particular, an antibody that identifies a novel subset of cerebellar cortical neurons.

Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.