Molecular Cloning of the Zeta Chain of the T Cell Antigen Receptor

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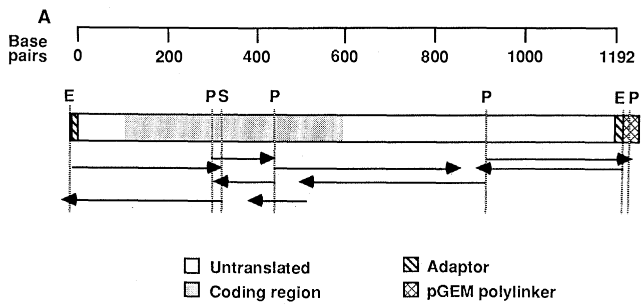
The T cell antigen receptor is a multi-subunit receptor complex present on the surface of all mature and many developing T cells. It consists of clonotypic heterodimers noncovalently linked to five invariant chains that are encoded by four genes and referred to as the CD3 complex. The CD3 gamma, delta, and epsilon chains have been molecularly characterized. In this report the molecular cloning of a complementary DNA encoding the zeta chain of the murine T cell antigen receptor is described. The predicted protein sequence of the zeta chain suggests a structure distinct from those of any of the previously described receptor subunits.

THE T CELL ANTIGEN RECEPTOR (TCR) performs two functions: the recognition of specific antigen and the translation of antigen binding into biochemical signals that activate the immune response of T cells [for review, see (*1*)]. Fulfillment of these functions requires the synthesis, assembly, and surface expression of a multi-subunit receptor complex. On most mature T cells, antigen recognition occurs via clonally specific alpha and beta chains that form disulfide-linked heterodimers and arise through gene rearrangement and other mechanisms (*2*). These clonotypic chains are noncovalently linked to a set of nonpolymorphic proteins in all T cells (*3*). These associated subunits, collectively referred to as the CD3 complex, consist of two glycoproteins, gamma and delta, and two nonglycosylated proteins, epsilon and zeta. The zeta chain is the most recently identified component of this complex in both murine and human T cells (*4*) and exists primarily as a disulfide-linked homodimer. Thus the receptor consists of a minimum of seven chains—alpha, beta, gamma, delta, epsilon, and two zetas. The complete sequences for human and murine gamma, delta, and epsilon subunits have been reported (*5*). The gamma and delta chains show extensive sequence similarity to each other and to the epsilon chain, and all three are members of the immunoglobulin supergene family (*6*). The genes encoding these three proteins are closely linked to each other in the genome (*7*).

The zeta chain has an apparent molecular size of about 16 kD when analyzed under reducing conditions by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). Approximately 90% of zeta exists as a disulfide-linked homodimer and, when nonreduced, migrates at 32 kD. There is evidence (*8*) that zeta also exists as a heterodimer disulfide linked to a unique 22-kD protein not previously described. In addition, our studies suggest that the receptor subunit that is tyrosine phosphorylated in response to antigen and that we previously termed p21 (*9*) is, in fact, tyrosine-phosphorylated zeta migrating anomalously on SDS-PAGE as a result of multiple phosphorylations.

Zeta plays a unique role in the assembly, intracellular targeting, and function of the TCR complex. Studies in murine T cell hybridomas indicate that zeta is synthesized in limiting amounts in comparison with the other subunits (*10*). Results of recent studies in a variant hybridoma cell line that fails to synthesize any detectable zeta chain (*11*) emphasize the unique role of zeta. In the absence of zeta, 95% of the receptor complexes are rapidly transported to and degraded in lysosomes, hence the zeta homodimer appears necessary for efficient cell-surface expression of the complex by preventing the sorting of the receptor from the trans Golgi to the lysosome. In the variant cells, only 5% of the pentameric complex reaches the cell surface. This partial complex (lacking zeta) is incapable of mediating a response to either specific antigen or to antibodies directed against those accessory surface molecules that can activate the parental cell, such as Thy-1. In order to further understand this important TCR component we have isolat-



B

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CAAAGCCAGCAGAGACTCCATCAGCGCCTCCTTTTCTCCTCATCCTCCCAGGCATAGCTG   60
                                         1
                                         MetLysTrpLysValSer
CCTCTGCCTCTGCCTCTGGGTACCATCCCAGGGAAGCAGAAGATGAAGTGGAAAGTGTCT  120
          10                            20   ↓
ValLeuAlaCysIleLeuHisValArgPheProGlyAlaGluAlaGlnSerPheGlyLeu
GTTCTCGCCTGCATCCTCCACGTGCGGTTCCCAGGAGCAGAGGCACAGAGCTTTGGTCTG  180
          30                            40
LeuAspProLysLeuCysTyrLeuLeuAspGlyIleLeuPheIleTyrGlyValIleIle
CTGGATCCCAAACTCTGCTACTTGCTAGATGGAATCCTCTTCATCTACGGAGTCATCATC  240
          50                            60
ThrAlaLeuTyrLeuArgAlaLysPheSerArgSerAlaGluThrAlaAlaAsnLeuGln
ACAGCCCTGTACCTGAGAGCAAAATTCAGCAGGAGTGCAGAGACTGCTGCCAACCTGCAG  300
          70                            80
AspProAsnGlnLeuTyrAsnGluLeuAsnLeuGlyArgArgGluGluTyrAspValLeu
GACCCCAACCAGCTCTACAATGAGCTCAATCTAGGGCGAAGAGAGGAATATGACGTCTTG  360
          90                           100
GluLysLysArgAlaArgAspProGluMetGlyGlyLysGlnGlnArgArgArgAsnPro
GAGAAGAAGCGGGCTCGGGATCCAGAGATGGGAGGCAAACAGCAGAGGAGGAGGAACCCC  420
         110                           120
GlnGluGlyValTyrAsnAlaLeuGlnLysAspLysMetAlaGluAlaTyrSerGluIle
CAGGAAGGCGTATACAATGCACTGCAGAAAGACAAGATGGCAGAAGCCTACAGTGAGATC  480
         130                           140
GlyThrLysGlyGluArgArgArgGlyLysGlyHisAspGlyLeuTyrGlnGlyLeuSer
GGCACAAAAGGCGAGAGGCGGAGAGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGC  540
         150                           160
ThrAlaThrLysAspThrTyrAspAlaLeuHisMetGlnThrLeuAlaProArgEnd
ACTGCCACCAAGGACACCTATGATGCCCTGCATATGCAGACCCTGGCCCCTCGCTAACAG  600
CCAGGGCATTTCTCCCTCACGGGCTTCACCTGCTGATGTCACTTGTGAAGGACAGAGGAC  660
AAAGCCCCCCTCAGTTTATTCATTTCCCAGCCACCATTTCATGACGAGGATGGTTCTCTC  720
ACTTGCCACATTTGTCTTCTTCAGTTCCAGAGCACTGAACACAGAACGTCATCCCTGGAC  780
TCTCTAAAGGGAGAGCCACCCTTGCTCTTCCACCCCAGCCCTGCTCTTGGGTCTTCTGGC  840
AGGCTCCTCTCCTTGCAGAGCCCAGCCCTAGCTAGGAGTTGGGGGTGGAGGGTGGGGCAC  900
TAACACACTCCCTCCTGCAGCTAGCTGAGTTCAGTTTGCTTTGTAAAGTCCCCAGAGAAG  960
CCCTAGGTACTGTGTGTATTGTTCTATGGGTATTGACTCGCTCCGCTCCTGCTGTAAATT 1020
TGGCTTCTGTTGTCACACTTTGCAGTGTTGAGGTAACATGTAATTAGGCCACATTGTGAA 1080
AGGCAGAGAGGCAGGTACAAGGGAGTCCAGGTAAATGCCAGCCAGAGGTGGCTCAAAGAA 1140
GGGAAGCAACACACAAGGAAGGTTCCTAGCCACAGGGGAACAGTAACAAGG          1191
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Fig. 1. (**A**) Single-stranded dideoxy chain termination sequencing was carried out on restriction fragments of the cDNA subcloned into bacteriophage M13 mp18 and M13 mp19 (*22*) using Sequenase (U.S. Biochemicals) and DNA polymerase I large fragment (BRL). Origins of arrows correspond to the restriction sites from which sequencing was initiated: P, Pst I; E, Eco RI; and S, Sst I. Arrows without restriction sites above them denote oligonucleotide priming based on previous sequence. (**B**) Complementary DNA sequence and deduced amino acid sequence. Arrow indicates predicted signal peptidase cleavage site. Underlined region corresponds to the transmembrane domain.

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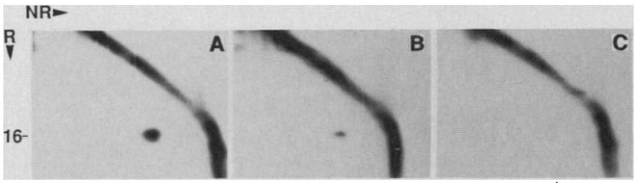

Fig. 2. Immunoprecipitation of iodinated membrane lysate from 2B4 cells. ^{125}I-labeled membrane lysates were prepared with NaI and lactoperoxidase as described (*4*) and immunoprecipitated with protein A–Sepharose (BRL) bound with the indicated antisera: (**A**) antiserum R124 raised in rabbit against purified zeta protein; (**B**) antiserum to zeta peptide; and (**C**) antiserum from unimmunized rabbit. Samples were eluted from Sepharose beads under nonreducing conditions and run in the first dimension (NR) on 10% SDS-PAGE gels (*4*). Tube gels were then equilibrated in sample buffer containing 0.5% dithiothreitol to reduce disulfide bonds and then run in the second dimension (R) on 12.5% SDS-PAGE slab gels. Gels were fixed, dried, and placed at −70°C for autoradiography as described (*5*). Zeta fell below the diagonal to 16 kD.

ed and cloned a complementary DNA (cDNA) encoding the murine zeta chain.

The hyperplastic lymph nodes of the MRL/lpr mouse (*12*) served as a plentiful source of T cells for zeta protein purification (*13*). Initial attempts at protein sequencing found the amino terminus of zeta to be blocked; hence purified zeta was subjected to cyanogen bromide (CNBr) cleavage and sequential Edman degradation. As CNBr cleavage yielded only two fragments by SDS-PAGE, and attempts to separate the peptides by high-performance liquid chromatography (HPLC) resulted in severe losses, we sequenced unfractionated CNBr cleavage products (*14*). Complementary nondegenerate 72-base oligonucleotides corresponding to a predicted stretch of 24 amino acids were synthesized (*14*). After purification these oligonucleotides were used to screen a T cell hybridoma (2B4) cDNA library (*15*). Four positive clones were purified, and each contained 1.25-kb inserts. Two of these inserts were cloned into the Eco RI site of the plasmid pGEM 3Z (Promega) and were shown to be identical by restriction enzyme analysis.

Nucleotide sequencing of the cDNA (Fig. 1) revealed an open reading frame encoding 164 amino acids before a single TAA stop codon was reached. Sequence 5′ to the presumed initiating ATG codon reveals a stop codon (TAG) in frame with the downstream open reading frame. The predicted protein sequence contained the amino acid sequence obtained by Edman degradation. However, the amino acid sequence used as a basis for oligonucleotide synthesis proved to have been a fusion of two CNBr-generated sequences (*14*). The deduced amino acid sequence predicts a protein with a molecular weight of 18,637. The amino terminus resembles a typical leader peptide. The sequence, analyzed according to the weight matrix method of von Heijne (*16*), confirmed the presence of a leader sequence, with the signal peptidase cleavage site located between residues 21 and 22. This would result in a mature protein of 143 amino acids with a predicted molecular weight of 16,299, in good agreement with the SDS-PAGE migration of zeta. Analysis of the predicted sequence of the mature protein (*17*) reveals a single hydrophobic stretch between residues 31 and 51. This would result in nine amino acids in the extracellular domain of the mature protein followed by a transmembrane helix of 21 amino acids. This transmembrane region is remarkable for the presence of a single negative charge (Asp) at residue 36. The cytoplasmic domain is predicted to contain 113 amino acids. Analysis of charged residues predicts a basic *p*I for the entire protein, in agreement with the known *p*I of zeta. A search of the National Biomedical Research Foundation database, March 1987 release by the FASTP program designed by R. Pearson and D. J. Lipman, revealed no significant homology between zeta and other known proteins. Notably, there was no significant homology between the zeta protein and the published sequences of the other CD3 components.

Various studies were carried out to determine whether the isolated cDNA clone indeed encoded the zeta subunit of the T cell receptor. A synthetic peptide, generated according to the protein sequence predicted by amino acid sequencing (*14*), was used to immunize rabbits. The resulting antiserum, R338, specifically precipitated the zeta homodimer from ^{125}I-labeled T cell membranes, although the titer was less than that obtained with antisera directed against purified zeta protein (*4, 10, 11*) (Fig. 2). The identity of the protein product encoded by the cDNA was further studied by in vitro transcription and subsequent translation in a rabbit reticulocyte lysate system either in the presence or absence of dog pancreas microsomes (Fig. 3). The resulting [^{35}S]methionine-labeled translation product was subjected to immunoprecipitation with two different sera (R123 and R124) raised against the purified zeta protein. In the absence of microsomes, a single major band with an apparent molecular size of 18,500 was precipitated specifically by these antibodies but not by an irrelevant antiserum. The antiserum to the zeta peptide (R338) did not recognize the in vitro translation product. When translation was carried out in the presence of microsomes, most of this 18-kD band was converted to a band with a molecular size of 16 kD (in agreement with the size of mature zeta). This product could again be immunoprecipitated with R123 and R124, the antisera specific for zeta (Fig. 3). This confirms that the predicted leader peptide is removed during the biosynthesis of the zeta chain.

The expression of the zeta chain gene was assessed by Northern blot analysis (Fig. 4); radiolabeled zeta cDNA was used as a probe. A single band of about 1.7 kb was seen in two T cell lines, EL4 and the 2B4 hybridoma (lanes 2 and 4 in Fig. 4). No hybridizing messenger RNA (mRNA) was detected from the B cell–derived G7 plasmacytoma cell line (lane 1). The B cell tumor line LK 35.2 showed faint hybridization (lane 3). Most significantly, the 2B4 variant 5.8 (*12*), which synthesizes all of the TCR components except zeta, contains no detectable mRNA for zeta (lane 5). Analysis of RNA isolated from mouse organs revealed high levels of expression in thymus and lower levels in spleen, with no detectable mRNA in brain, liver, kidney, heart, or cultured fibroblasts. Several other B and

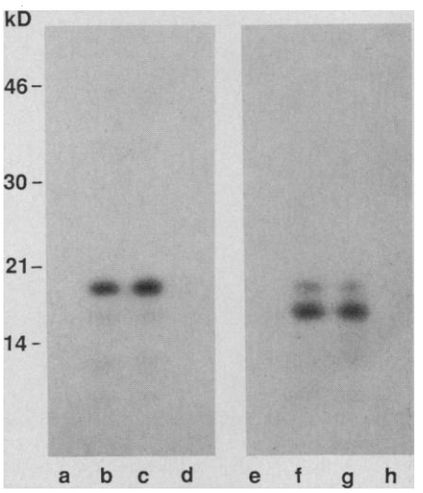

Fig. 3. In vitro translation of cDNA transcript. Plasmid pGEM3Z containing the intact cDNA was linearized and transcribed with T7 polymerase (Promega). In vitro translation was carried out according to the manufacturer's suggestions with nuclease-treated rabbit reticulocyte lysate (Promega) with [^{35}S]methionine without (lanes a to d) or with (lanes e to h) added canine microsomal membranes. After 1 hour of translation at 30°C, the reaction mixture was lysed in 0.5% Triton X-100, 300 m*M* NaCl, and 50 m*M* tris, *p*H 7.6. Immunoprecipitation was then carried out with (lanes a and e) a control antiserum (to the CD3 delta peptide), (lanes b and f) antiserum R123, (lanes c and g) antiserum R124, or (lanes d and h) antiserum R338 (to the zeta peptide). Thirteen percent SDS-PAGE gels were run, fixed, treated with 1*M* sodium salicylate (intensifying fluor), and dried before autoradiography.

pre-B cell lines were similarly analyzed and no detectable message was seen (*18*). Thus the cDNA specifically hybridizes with an mRNA present primarily in T cells and absent in a 2B4 variant that specifically fails to synthesize zeta.

A model for the zeta homodimer based on the deduced amino acid sequence (Fig. 5) shows several notable features. First, there is a single cysteine, which is present on the external face of the transmembrane region. Hence, all disulfide interactions between zeta and itself or other polypeptide chains must occur through the same residue. Second, the transmembrane region of the protein contains a single negative charge; the finding of charged amino acids in transmembrane domains is unusual; yet all of the

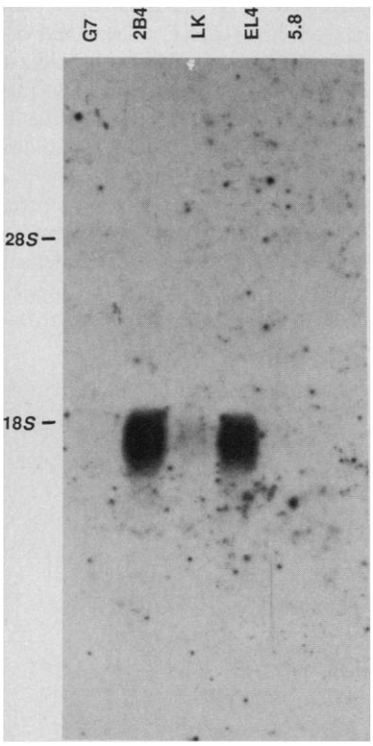

Fig. 4. RNA was prepared from cultured cells (*23*), and polyadenylated [poly(A)] mRNA was selected with oligo(dT) cellulose (New England Biolabs). After electrophoresis in 0.7% agarose gels, Northern blotting of RNA to nitrocellulose was carried out. Filter hybridization was carried out in 50% formamide, 5× SSPE, 5× Denhardt's solution (*23*), 0.1% SDS, yeast transfer RNA (200 μg/ml), and 10% dextran sulfate (Pharmacia). Radiolabeled probe was generated by oligo priming (Pharmacia) of purified zeta cDNA. The film was washed at 22°C in 2× SSC (1× SSC is 0.15*M* NaCl and 0.015*M* sodium citrate) and then at 65°C in 0.1× SSC and 0.1% SDS prior to autoradiography at −70°C. Equal amounts of poly(A)-selected material were loaded in each lane except for the G7 plasmacytoma cell line in which nonselected material was loaded. In the G7 lane, four times as much total RNA was loaded, and subsequent rehybridization of the filter with an actin probe demonstrated that the amount of mRNA in the G7 lane was equivalent to that in the other lanes.

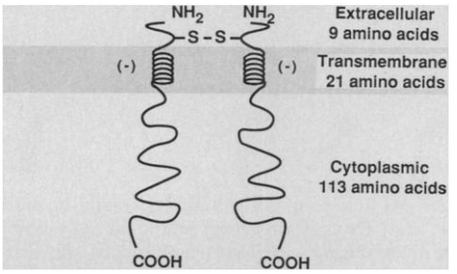

Fig. 5. Schematic model of disulfide-linked mature zeta protein in the cell membrane.

CD3 components have similarly placed single negative charges. It is interesting that all of the clonotypic subunits (including the more recently described rearranged TCR gamma and delta chains) (*2, 19*) have single charged residues in their transmembrane domains; however, these residues are positively charged. The existence of this unusual feature in this multi-subunit structure raises the possibility that these charged residues play a role in receptor assembly and stabilization. Third, in terms of mass distribution, the zeta subunit has the smallest extracellular domain of all the receptor components and, unlike the other CD3 components, it does not have an external immunoglobulin-like domain. With respect to the membrane, the zeta homodimer is a mirror image of the alpha-beta heterodimer. The alpha-beta heterodimer contains fewer than ten intracytoplasmic amino acids and has large extracellular domains consistent with its known function in the recognition of external antigen-MHC complexes. In contrast, the structure of the zeta homodimer with its small extracellular domain and large cytoplasmic tail is consistent with evidence suggesting it has a primary role in signal transduction. This cytoplasmic domain contains six potential sites for tyrosine phosphorylation. A fourth feature of the deduced amino acid sequence is the presence of a consensus sequence (*20*) for adenosine triphosphate (ATP) binding (Gly-x-Gly-x-x-Gly-x-x-x-Gly . . . -Ala-x-Lys) between residues 135 and 150; this represents one variant of this consensus sequence The presence of this motif is of particular interest given the fact that zeta, or a protein to which it is disulfide-linked, undergoes tyrosine phosphorylation in response to antigenic stimulation. Whether zeta actually binds ATP, what its actual role is vis-à-vis antigen-induced tyrosine phosphorylation, and how it might function to couple external recognition to intracellular signaling are questions for further investigation.

Note added in proof: We have recently cloned and sequenced cDNAs encoding the human zeta chain (*21*). The deduced protein sequence shows 87% homology to the murine cDNA.

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13. For protein purification, lymph nodes were harvested from MRL/lpr mice and cells were dispersed. Red blood cells were lysed in 0.14*M* NH_4Cl and 0.017*M* tris, *p*H 7.2. After cells were washed in phosphate-buffered saline (PBS), they were suspended in 10 m*M* tris-HCl, *p*H 7.6, and 0.5 m*M* $MgCl_2$ in the presence of protease inhibitors. The cells were then lysed in a nitrogen cavitation device (Parr) at 10.5 kg/cm^2 pressure. The tonicity of the suspension was restored with 600 m*M* NaCl, and phosphatase inhibitors were added. The nuclear fraction was removed by centrifugation at 300*g* for 10 minutes. Membranes were prepared, lysed, immunoprecipitated, eluted from antibody, and concentrated; radiolabeled tracer was then added as described (*4*) except that affinity-purified antibodies raised in goat against the carboxyl terminus of the murine delta chain were used for immunoprecipitation. We previously described similar antibodies raised in rabbits (*24*). SDS-PAGE gels (*4*) were run first under nonreducing conditions in tubes and after reduction were run in the second dimension. After autoradiography, reduced zeta falling below the diagonal was identified and gel slices were cut out for elution and sequencing. Recrystallized SDS was used throughout, and sodium thioglycolate was added to the running buffer.
14. Sequencing was carried out on 100 p*M* unfractionated, CNBr-cleaved, purified zeta by Edman degradation. The sequence obtained was Ala-Glu-Ala-Tyr-Ser-Glu-Ile-Gly-Asn-Pro-Gln-Glu-Arg-Val-Tyr-Asn-Ala-Leu-Gln-Lys-Asp-Lys-Tyr (a Met at the amino end was assumed). A 72-base oligonucleotide was constructed on a DNA synthesizer (Applied Biosystems model 301) with the following 5′ to 3′ sequence: ATGGCTGAGGCCTACTCTGAGATTGGCAACCCCCAGGAGCGGGTCTACAATGCCCTGCAGAAGGACAAGTAC. Its complement was also synthesized. Best-guess codons were chosen according to the codon usage rules of Lathe (*25*). Examination of the deduced amino acid sequence used for oligonucleotide and peptide synthesis re-

vealed it to have been a fusion of two CNBr cleavage fragments, one beginning at amino acid 97 and the other beginning at amino acid 120 and put together in the following order: Ala-120 to Gly-127, Asn-105 to Glu-108, Arg-132, Val-110 to Lys-118, and Tyr-142. All of the amino acids are in the proper cycle of Edman degradation chemistry, with Met-96 and Met-119 as the points of chemical cleavage. A sequence with 100% identity to amino acids 96 to 104 was seen on sequencing but was interpreted as a minor sequence.
15. A cDNA library constructed from the murine helper cell line 2B4 in λgt10 was a gift from David Cohen. Screening was carried out with the two complementary 72-base oligonucleotides. Nitrocellulose filters containing phage DNA were prehybridized overnight and then hybridized in 30% formamide (Fluka), 5× SSPE (1× SSPE is 0.18*M* NaCl, 0.01*M* sodium phosphate, *p*H 7.7, and 0.01*M* EDTA), 5× Denhardt's solution, yeast RNA (200 μg/ml) (Calbiochem), salmon sperm DNA (100 μg/ml) (Sigma), and 10% dextran sulfate (Pharmacia) at 42°C. Synthetic probes were radiolabeled by addition of ^{32}P to the 5′ end with the use of T4 polynucleotide kinase (Boehringer Mannheim) and [γ-^{32}P]ATP (Amersham). Filters were subjected to serial washings in 2× SSC at 22°C, then at 0.2× SSC and 0.1% SDS at 42°C. Screening was repeated until plaque purification was obtained. Clonal phage DNA was prepared with Lambdasorb (Promega) according to the manufacturer's instructions. Inserts were purified out of agarose gels with DEAE membrane (Schleicher and Schuell) according to manufacturer's instructions. Purified insert was cloned into pGEM3Z (Promega).
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Second Conserved Domain of gp120 Is Important for HIV Infectivity and Antibody Neutralization

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Rabbit antisera were raised against three overlapping synthetic peptides with sequence homology to the second conserved domain of the external envelope glycoprotein (gp120) of the human immunodeficiency virus (HIV). All of the antisera immunoprecipitated the envelope glycoprotein. In particular, an antiserum directed against amino acids 254 to 274 of *env* was efficient in neutralizing three different isolates of HIV in vitro, without affecting the binding of the virus to CD4-positive cells. Therefore, this conserved region of gp120 appears to be critical in a postbinding event during virus penetration and may represent a target for antibody neutralization of HIV. These findings may be applicable in the design of a vaccine for the acquired immunodeficiency syndrome.

THE SEQUENCE OF THE *env* GENE varies greatly among different isolates of the human immunodeficiency virus (HIV), but this variation is not randomly distributed throughout the gene (*1*). Instead, a pattern of alternating variable and constant regions is observed (*2*). In the external envelope glycoprotein (gp120), several constant and variable domains have been identified. In the constant domains, sequence conservation is greater than 80% among different isolates of HIV, whereas in the most variable domains, sequence conservation is between 20 and 30%. Conservation of sequence within the constant domains presumably indicates selective pressure to maintain those sequences, although their functional importance is not understood.

The second conserved domain of gp120 is partially homologous to neuroleukin, a factor that has both neurotrophic and lymphokine activities (*3*). We found earlier that gp120 inhibits the neurotrophic action of neuroleukin on cultured neurons, and this finding may be important for understanding the pathogenesis of dementia observed in some patients with acquired immunodeficiency syndrome (AIDS) (*4*). To determine whether the second conserved domain is important for HIV infectivity, we prepared antisera against synthetic peptides with sequences homologous to that region of gp120. We now report that one of these antisera is strongly immunoreactive with gp120 and is also highly neutralizing for diverse isolates of HIV. However, this antiserum does not block the binding of HIV to $CD4^+$ cells. These findings suggest that one particular region, comprising amino acids 254 to 274 within the second conserved domain of gp120, takes part in a postbinding event during virus penetration and is an important target for antibody neutralization of HIV.

Three overlapping oligopeptides—designated T19V, C21E, and S19C—were synthesized in accordance with the amino acid sequence of the HTLV-III_B isolate (*1, 5*) of HIV (Fig. 1). Each peptide was coupled to keyhole-limpet hemocyanin (KLH) and used to prepare antisera in rabbits. The titer and specificity of the three antisera were determined by enzyme-linked immunosorbent assay (ELISA). Each of the rabbits had a strong antibody response to its immunizing peptide but not to a control peptide, M20L (Table 1). The antiserum to C21E (anti-C21E) also reacted with the peptide that overlaps C21E at its amino terminus (T19V), but not with the peptide that overlaps at its carboxyl terminus (S19C). Anti-T19V showed little cross-reactivity with C21E; anti-S19C showed no cross-reactivity with C21E (Table 1).

All three of the antisera immunoprecipitated proteins of 120 or 160 kD, or both, from Molt-III cells infected with the HTLV-III_B isolate (Fig. 2, lanes b). These proteins were not immunoprecipitated from uninfected

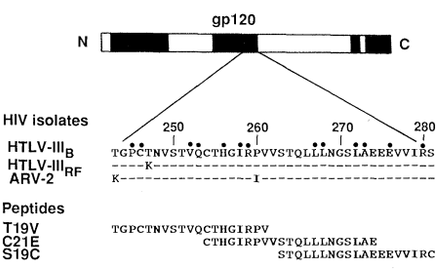

Fig. 1. The amino acid sequences of HTLV-III_B, HTLV-III_{RF}, and ARV-2 were obtained (*1, 2*), and the numbering system was based on that of Ratner *et al.* (*1*). The conserved regions of gp120 are shown in black. A dash indicates identity with the HTLV-III_B sequence, and a dot denotes the amino acids that are homologous to neuroleukin (*3, 4*). The three peptides—T19V, C21E, and S19C—were synthesized by Peninsula Laboratories, Inc. (Belmont, California), and correspond to the sequence of HTLV-III_B; their composition was confirmed by amino acid analysis.

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