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Natural Catalytic Immunity Is Not Restricted to Autoantigenic Substrates

Identification of a Human Immunodeficiency Virus gp120-Cleaving Antibody Light Chain

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Abstract

The autoimmune repertoire is well known from previous studies to be capable of producing catalytic antibodies directed to self-antigens. In the present study, we explored the ability of 26 monoclonal light chains (Lchains) from multiple myeloma patients to cleave radiolabeled gp120, a foreign protein. One L chain with this activity was identified. ^{125}I-gp120 and unlabeled gp120 were cleaved at several sites by the L chain, as shown by SDS-polyacrylamide gel electrophoresis, autoradiography, and immunoblotting, respectively. The apparent dissociation constant of the L chain was 130–145 n*M*, indicating high-affinity gp120 recognition. ^{125}I-albumin was not cleaved by the L chain, and various proteins and peptides did not inhibit gp120 cleavage by the L chain, suggesting that the activity is not a nonspecific phenomenon. The substrate recognition determinants may be conserved in different HIV-1 strains, because gp120 isolated from strains SF2, MN, and IIIB was found to be cleaved by the L chain. Micromolar concentrations of a synthetic peptide corresponding to residues 23–30 of gp120 inhibited the cleavage of ^{125}I-gp120, suggesting that these residues are components of the epitope recognized by the L chain. The toxic effect of gp120 in neuronal cultures was reduced by about 100-fold by pretreatment of the protein with the L chain. These observations open the possibility of utilizing gp120-cleaving antibodies in the treatment of AIDS.

Index Entries: HIV-1; AIDS; gp120; catalytic antibody; light chain.

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Introduction

Antibodies with proteolytic activity offer a potent source of immunological defense against microbial disease. If the product fragments generated by antibody-catalyzed cleavage of the target polypeptide are biologically inactive, permanent inactivation of the target polypeptide can be anticipated. Moreover, if the antibodies are specific for individual polypeptide substrates, collateral damage due to nonspecific proteolysis can be avoided. However, it is unknown whether the immune system actually draws upon antibody catalysis to achieve protection against microbes. Most previous reports on antibody catalysis have focused on the ability of certain autoantibodies to utilize autoantigens as substrates, e.g., vasoactive intestinal polypeptide (VIP) *(1–3)*, thyroglobulin *(4)*, vasopressin *(5)*, DNA *(6)*, and RNA *(7)*. For this reason, it has been widely assumed that some special dysfunction characteristic of autoimmune disease might be responsible for the synthesis of catalytic antibodies.

In the study reported here, we searched for monoclonal light chains (L chains) from multiple myeloma patients with the ability to cleave gp120. One of 26 L chains examined was observed to cleave gp120. The reaction was characterized by comparatively high-affinity gp120 recognition: gp120 preparations from three viral strains were cleaved by the L chain, and gp120 digested with the L chain displayed reduced neurotoxicity in a cell-culture model. We conclude that antibody proteolytic activities are not limited to classical autoantigens and classical autoimmune disease. Previously, proteolytically active L chains have been identified in nonautoimmune mice following immunization with an autoantigenic peptide (VIP-carrier conjugate) *(8)* and an HIV peptide (a gp41 peptide-carrier conjugate) *(9)*.

gp120 bound to gp41 on the surface of HIV-1 particles is believed to initiate viral infection by binding host-cell receptors *(10)*. Soluble gp120 shed from the virus is believed to exert cytotoxic effects on neurons and blood cells *(11)*. Our findings pave the way toward development of the antibody catalysts for immunotherapy of AIDS.

Materials and Methods

L Chains

Monoclonal L chains purified to electrophoretic homogeneity from the urine of multiple myeloma patients *(12)* were supplied by Dr. Alan Solomon (University of Tennessee) and Dr. Fred Stevens (Argonne National Laboratory). These were analyzed for the gp120 cleavage activity (laboratory designations for the L chains: B6, Bor, Bos, Bos, Cag, Col, Cro, Eve, Gal, Fre, Has, Hou, Joh, Mcp, Mor, Laym, Len, Rhe, Rhy, Rob, Pat, Tew, Und, Wat, Wit, Xoc). The test set included κ and λ L chains of various subgroups. Amino-acid sequencing of the Laym L-chain tryptic peptides has

shown them to belong to the κII subgroup (Dr. A. Solomon, unpublished). SDS-electrophoresis and immunoblotting was done as in *(2)* using rabbit antihuman κ chain antibodies for staining (1:100, reactive with bound and free L chains; Axell).

Radiolabeled gp120 Cleavage

Recombinant gp120 preparations expressed in Chinease hampster ovary (CHO) cells (strain SF2) or in a baculovirus system (strains IIIB and MN) were from the NIH AIDS Research and Reference Reagent Program and Agmed, respectively. The gp120 (5 μg) was labeled with $Na^{125}I$ (Amersham, 1 mCi) using chloramine-T (24 μg) in 75 μL of 0.1 *M* sodium phosphate buffer, pH 7.4, for 30 s. Sodium metabisulfite (120 μg) was added, and free ^{125}I was removed by gel filtration on an EconoPac 10 DG column (BioRad) in 50 m*M* sodium phosphate, 0.5 *M* sodium chloride, 0.1 m*M* CHAPS, and 0.02% sodium azide, pH 7.4 (PBS-CHAPS). The radiolabeled protein recovered at the void volume was stored at –80°C in aliquots. SDS-electrophoresis on Phast gels (8-25%, Pharmacia) and autoradiography (Kodak XAR-5 film) showed a major radiolabeled band at 120 kDa, corresponding to intact gp120. In some preparations, a minor 90 kDa band was also evident, which is presumably a breakdown product of gp120 present in the original gp120 preparation. Cleavage of gp120 was determined by incubation of the radiolabeled protein (about 4×10^5 cpm, corresponding to 2 n*M* gp120) with the L chains in 20 μL PBS-CHAPS 37°C. The reaction was terminated by addition of 5 μL electrophoresis buffer containing 10% SDS, the samples subjected to SDS-electrophoresis and autoradiography, and the areas of bands evident on autoradiograms determined using an image analyzer *(4)*. Because of the high affinity of the L chain for gp120 and its slow cleavage rate, it was necessary to keep the L-chain concentration greater than the gp120 concentration for study of reaction kinetics. Under these conditions, total and free substrate concentrations are not equivalent, and application of the Michaelis–Menten equation is subject to error. Therefore, the apparent kinetic constants K_s and V_{max} were computed by the method of Smith et al. *(13)*, which is valid regardless of the relative concentrations of the substrate and the L chain. The cleavage of ^{125}I-gp120 (4.2 n*M*) incubated with the Laym L chain (75 n*M*) for increasing lengths of time (12, 16, 24, and 32 h) was measured in preliminary assays. Substrate depletion was observed to be a linear function of incubation time. Since the rate of substrate depletion is constant, pseudo-steady-state conditions prevail, and the Smith method can be assumed to be a valid means to estimate the kinetic constants. Polypeptides tested as inhibitors of ^{125}I-gp120 cleavage were albumin (Sigma), calmodulin (Sigma), neurotensin (Peninsula), bombesin (Peninsula), secretin (Bachem), hGRF (Hoffman-LaRoche), and the synthetic MN gp120 peptide set available from the NIH AIDS Research and Reference Reagent Program. The gp120 peptide set consisted of 43 synthetic overlapping 20-mer peptides, one

8-mer peptide (residues 23–30 of gp120), and one 9-mer peptide (residues 27–35), spanning residues 1–520 of the envelope protein, with 10 amino-acid overlaps between the sequential 20-mer peptides. Certain 20-mer sequences were not available because of poor solubility or technical difficulties (residues 11–30, 21–40, 51–70, 71–90, 191–210, 371–390, 411–430, and 441–460). The 8-mer and 9-mer peptides represent the available segments of residues 11–30, and decapeptide sequences from the unavailable 20-mers are found in the adjacent overlapping peptides that were tested as possible inhibitors. Radiolabeling of albumin (RIA grade, Sigma) and assay of ^{125}I-albumin cleavage was conducted by the methods described for gp120.

Unlabeled gp120 Cleavage

gp120 (40 μg; SF2) was incubated with 15 μg L chain in 30 μL PBS-CHAPS for 24 h. The reaction conditions and SDS-electrophoresis procedure were as described for radiolabeled gp120. Product fragments were visualized by treatment of blots of the gels with rabbit antiserum to gp120 (1:500 for 18 h; Biogen, Cambridge, MA) described to recognize proteolytic fragments of gp120 electrophoresed under reducing conditions *(14)*, peroxidase conjugated goat antibodies to rabbit IgG (1:1000, 1 h; Cappel) and metal-enhanced diaminobenzidine solution (Pierce).

Neuronal Cell Cultures

To test for gp120 neurotoxicity, dissociated cerebral cortical cultures were prepared from newborn rats as previously described *(15)*. Briefly, brain tissue was dissociated with 0.25% trypsin and triturated. The cell suspension (5×10^5) was seeded on a confluent layer of cerebral cortical astrocytes in 35-mm dishes. In this preparation, all neurons are postmitotic. Cultures were maintained in 5% horse serum in minimal essential medium supplemented with defined medium components *(16)*. A complete change of medium was performed prior to treatment with gp120. Only one treatment with gp120 (IIIB strain) was done during the 5-d test period. Dilutions of the gp120 were made with phosphate-buffered saline. At termination, neurons were fixed and identified immunocytochemically with antiserum to neuron-specific enolase by methods previously described *(17)*. Neuronal cell counts were determined from 30 fields at predetermined coordinate locations. All samples were coded and counted without knowledge of their experimental identity. Statistical comparisons were made by a one-way analysis of variance with Student–Newman–Keul's multiple comparison-of-means test.

Results and Discussion

gp120 Cleaving L Chain

Previously reported studies have indicated that some monoclonal L chains isolated from multiple myeloma patients are capable of hydrolyz-

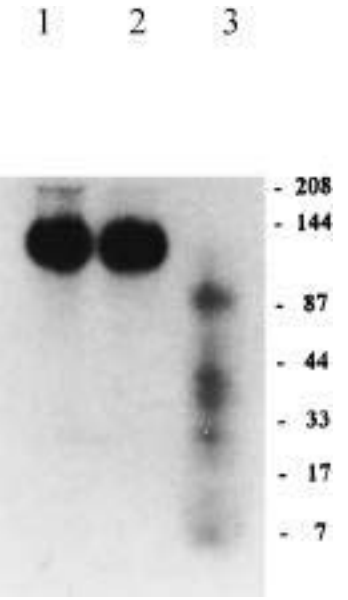

Fig. 1. Cleavage of ^{125}I-gp120 incubated with Laym L chain. Autoradiogram showing nonreducing SDS-electrophoresis gels of SF2 ^{125}I-gp120 (1.1 n*M*) incubated for 15 h (37°C) in assay diluent (lane 1), a noncatalytic L chain (2 μ*M*, code FRE; lane 2) and the Laym L chain (2 μ*M*; lane 3).

ing the autoantigenic neuropeptide VIP and certain synthetic protease substrates *(5,18,19)*. In our study, one of 26 monoclonal L chains screened at a concentration of 2 μ*M* displayed the ability to hydrolyze ^{125}I-gp120, which was evident as depletion of the 120 kDa intact ^{125}I-gp120 band and appearance of bands with lower molecular mass in nonreducing electrophoresis gels (87, 37, 24, 14, and approx 7 kDa) (*see* Fig. 1; designation of the gp120-cleaving L chain, Laym). In reducing gels, a reduction in the intensity of the 87 kDa product band, appearance of a band at about 64 kDa and an increase in intensity of lower-molecular-weight bands was evident, suggesting that the 87 kDa band is a complex of smaller, disulfide-bonded polypeptides, similar to the results observed for certain polypeptide complexes observed following digestion of gp120 with trypsin *(14)*. To eliminate the possibilities that radiolysis or structural changes induced by radiolabeling are responsible for the observed reaction, unlabeled gp120 was treated with this L chain and a blot of the reaction products electrophoresed under reducing conditions was stained with an anti-gp120 antibody known to recognize various fragments of gp120 *(14)*. Antibody-stainable gp120 product bands with estimated mass of 87, 64, 37, 24 and 15 kDa were evident (*see* Fig. 2). The cleavage profile of the unlabeled gp120 revealed by immunostaining corresponds to that of ^{125}I-gp120 determined by reducing electrophoresis, except that the 7-kDa band was undetected by the former procedure, probably reflecting the absence of an antibody-

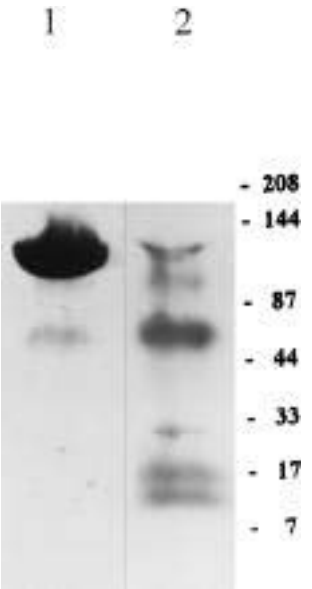

Fig. 2. Immunoblot showing cleavage of unlabeled gp120 by Laym L chain. gp120 (11.1 μ*M*) was incubated (24 h, 37°C) in assay diluent (lane 1) or the L chain (20 μ*M*; lane 2) and subjected to reducing SDS-gel electrophoresis. Staining of the blot was with rabbit antibody to gp120.

reactive epitope in this product fragment. Staining of the standard proteins used for mass calibration (myosin, β-galactosidase, bovine serum albumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme, and aprotinin) and the Laym L chain was not evident, indicating the specificity of the antibody for gp120 and its fragments.

Previous reports have shown that protease activity of monoclonal L-chain preparations is not due to adventitious proteins *(18,19)*. Evidence that the gp120-cleaving activity is due to the Laym L chain is as follows: 1) This L chain was the only one of 26 equivalently purified L chains studied to display the activity. 2) The activity was retained in the renatured 25 kDa monomer fraction of the L chain prepared by gel filtration in 6 *M* guanidine hydrochloride activity as in *(18)* (not shown). As discussed previously *(18,24)*, the denaturing gel filtration procedure precludes the possible involvement of protease contaminants bound noncovalently to the L chain. 3) SDS-electrophoresis of overloaded Laym L chain failed to reveal silver-stained proteins that were nonreactive with antihuman L chain performed as described previously *(2)*. 4) Sequencing of 10 *N*-terminal residues in the Laym preparation performed as in *(8)* yielded a single peptide sequence (Glu-Asp-Ile-Val-Met-Thr-Gln-Thr-Pro-Leu) corresponding to a κII subgroup L chain, with no evidence for the presence of contaminating sequences. 5) More important, the observed gp120-cleaving characteristics, i.e., high-affinity gp120 recognition and specificity for this protein, are

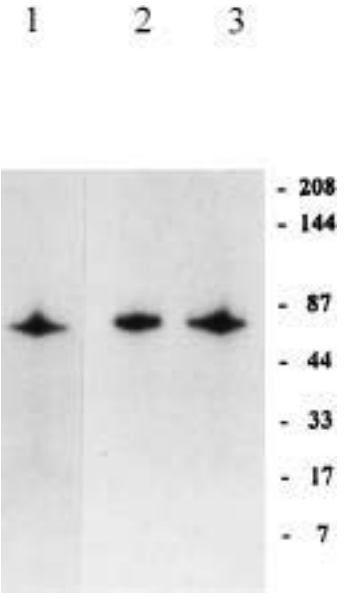

Fig. 3. Specificity of Laym L chain: Lack of cleavage of ^{125}I-albumin. Autoradiogram of nonreducing SDS-PAGE gel showing ^{125}I-albumin (1.4 n*M*) incubated (15 h, 37°C) in assay diluent (lane 3), a noncatalytic L chain (2 μM FRE; lane 2) and the gp120-cleaving Laym L chain (2 μM; lane 1).

typical of an antibody catalyst, and inconsistent with an involvement of conventional proteases.

Affinity and Specificity

Cleavage of ^{125}I-albumin by the Laym L chain was not detected (*see* Fig. 3). Unlabeled albumin at concentrations up to 10 μM did not inhibit the ^{125}I gp120 hydrolysis reaction by the L chain. Similarly, various peptides and polypeptides tested at a concentration of 10 μM (bombesin, secretin, helodermin, neurotensin, human-growth-hormone-releasing factor, and calmodulin) did not detectably inhibit the cleavage. These observations indicate that the Laym-mediated hydrolysis of gp120 is not a nonspecific reaction.

The rate of the cleavage reaction was saturable at increasing concentrations of gp120. Values of the apparent dissociation constant (K_d) and maximal velocity at saturating concentrations of gp120 from strain MN (V_{max}) were estimated at 130 n*M* and 1.8 n*M* gp120/20 n*M* L chain/30 h, respectively. The comparatively low K_d value suggests high-affinity gp120 recognition. L chains and antibodies capable of binding other polypeptide substrates with affinity comparable to Laym-gp120 recognition reaction have been described previously *(1,4,6)*. In comparison, there was no evidence for saturability of trypsin-catalyzed hydrolysis of gp120 at the highest gp120 concentration tested (0.3 μ*M*), consistent with comparatively low-affinity recognition of gp120 by this enzyme.

Since the sequence of gp120 is not conserved in different HIV-1 strains, antibodies can bind gp120 isolated from the different strains to variable levels. In the present study, gp120 preparations isolated from three different HIV-1 strains (SF2, IIIB, MN) were examined as the substrates for the L chain. The cleavage profiles of ^{125}I-gp120 from these strains visualized by SDS-electrophoresis following digestion with the L chain were identical. Quantitative scanning of the bands showed that the rates of digestion of ^{125}I-gp120 (expressed as percentage of available substrate per h) from strains MN, SF2, and IIIB by the L chain (0.25 μ*M*) were 10.0%, 3.1%, and 8.3%, respectively. The K_d of SF2 gp120 cleavage determined as in Fig. 4 was 145 n*M*, which is close to the value observed for MN gp120 (130 n*M*). The V_{max} for SF2 gp120 cleavage was 0.9 n*M*/75 n*M* L chain/32 h. These observations suggest that the gp120 epitope recognized by the L chain may be conserved in different HIV-1 strains.

Forty-five synthetic gp120 peptides were examined as possible inhibitors of gp120 hydrolysis by the L chain. These peptides spanned the sequence of gp120 residues 1–520. All but one of the synthetic peptides tested at a concentration of 5 μ*M* (which was about 2000-fold higher than the concentration of the gp120 substrate) had no effect on the rate of cleavage of ^{125}I-gp120 by the L chain. The inhibitory peptide is an 8-mer representing residues 23–30 of gp120 from strain MN (Leu-Leu-Met-Ile-Cys-Ser-Ala-Thr), which are largely conserved in different isolates of HIV-1. Measurement of inhibition of ^{125}I-gp120 cleavage at varying concentrations of this peptide yielded an IC50 value (6.5 μM) that was about 50-fold greater than the apparent K_m of the L chain, suggesting comparatively low-affinity inhibitor recognition. The 8-mer peptide could serve as an alternate substrate or as a competitive inhibitor that is not cleaved by the L chain. Regardless of the mechanism of the inhibition, it is evident that the peptide does not constitute the complete gp120 epitope recognized by the L chain with high affinity, and the native folded conformation of gp120 may be important in high-affinity recognition by the L chain. Additional structural components of the epitope must be spatial neighbors of the 8-mer peptide as expressed in gp120, but they do not need to be contiguous to this peptide in the linear sequence. The importance of conformational gp120 epitopes in the binding by antibodies has been noted previously *(21)*.

gp120 Neurotoxicity

As reported previously *(15)*, gp120 produced significant reduction ($p < 0.001$) in neuronal survival at ≥ 10^{-13}*M*. Pretreatment of gp120 with the Laym L chain resulted in a significant increase in neuronal survival ($p < 0.001$). The gp120 concentrations displaying half-maximal neurotoxicity were 3 p*M* and 0.03 p*M* following incubation of the protein with the Laym L chain and assay diluent, respectively. Electrophoresis of an aliquot of the gp120-L chain reaction mixture showed that about 90% of the gp120

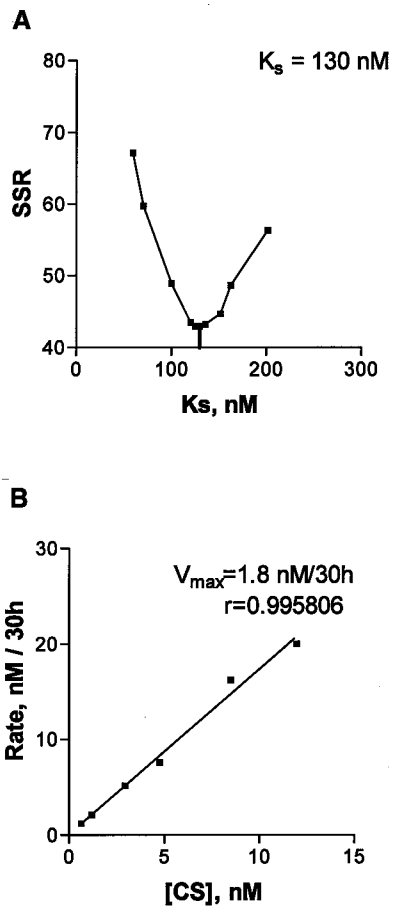

Fig. 4. Kinetic parameters for Laym-mediated gp120 cleavage: Plots of assumed K_s vs sum of square residuals (SSR) **(A)** and observed velocity vs [CS] at the K_s value yielding the least SSR value **(B)**. [CS] is the concentration of the catalyst–substrate complex. Cleavage rates were obtained by measuring the decrease in intensity of the ^{125}I-gp120 band following incubation of the L chain (20 n*M*, 37°C, 30 h) with increasing unlabeled gp120 concentrations (5 n*M*–200 n*M*) mixed with a fixed concentration of MN ^{125}I-gp120 (4.3 n*M*). [CS] values were computed at a series of assumed K_s values from the quadratic equation: $[CS]^2 - [CS]([C_1] + [S_1] + K_s) + ([C_1][S_1] = 0$, where $[C_t]$ and $[S_t]$ are the initial catalyst and gp120 concentrations. The best fit between observed rate and [CS] at each assumed K_s value was obtained by linear regression. The assumed K_s value at which the lowest sum of square residual SSR was observed in (*A*) corresponds to K_s of the L chain.

had been digested. No effect of the L chain on neuronal cell counts was observed in the absence of gp120.

Comment

Human autoantibodies have previously been shown to cleave polypeptide and nucleic acid substrates *(1–8)*, and mouse strains with a genetic

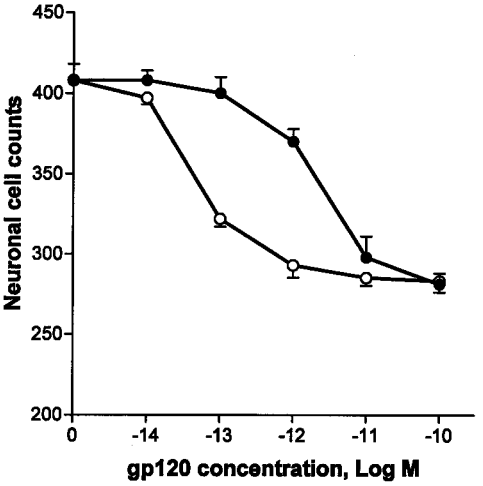

Fig. 5. Reduced neurotoxicity of gp120 treated with Laym L chain. gp120 from HIV-1 strain IIIB (initial concentration: 100 n*M*) was incubated in RPMI-1640 in the absence (○) or presence of LayM (10 μ*M*; ●) for 30 h at 37°C. Dissociated cerebral cortical neurons from newborn rats were treated with increasing concentrations of the reaction mixture corresponding to the gp120 concentrations plotted on the *x*-axis. Neuronal cell counts were determined by microscopy (see ref. *15*). Each value is the mean of four or five determinations made from two separate experiments. The error bar is the standard error.

proclivity to autoimmune disease display an increased tendency toward catalytic antibody synthesis *(22)*. Observations of cleavage of gp120 by the monoclonal L chain in the present study suggest that the phenomenon of antibody proteolysis is not restricted to autoantigens. Furthermore, autoimmune abnormalities are not known to play a role in defining the specificity or the maturation of the B cells that eventually become cancerous plasma cells in multiple myeloma patients (the source of our gp120-cleaving L chain). It may be concluded that the gp120-cleaving activity does not arise by way of a classical autoimmune response. It remains unclear, however, whether antibodies can develop proteolytic activity specifically in response to microbial infection. There is no evidence that the donor of the Laym L chain was infected with HIV-1 (the patient died before AIDS was a recognized clinical entity). It could be hypothesized that the gp120-cleaving activity is caused by a structural similarity between the inciting antigen and the gp120. However, we are unable to distinguish whether the inciting antigen might be a foreign protein or an autoantigen. Examples of foreign proteins *(23)* and autoantigens *(24)* with limited structural similarity to gp120 are abundant.

The ability of the Laym L chain to recognize its substrate (gp120) with high affinity and cleave this protein at multiple sites is analogous to the behavior of catalytic antibodies to VIP *(2)* and thyroglobulin *(4)*. These activities can be explained by the "split-site" mechanism *(25)*, in which

the "binding" subsite responsible for the initial high-affinity recognition of gp120 is distinct from the chemically reactive subsite responsible for peptide-bond cleavage. Stated in terms of catalysis theory *(26)*, the initial high-affinity binding corresponds to substrate ground-state stabilization and the peptide-bond cleavage reaction corresponds to transition-state stabilization. The validity of this model is supported by observations that 1) loss of the peptide bond-cleaving activity without loss of the high-affinity binding activity, and loss of the latter activity without loss of the former activity, can be induced by single amino-acid mutations in a proteolytic L chain *(27)*; and 2) substrate epitope components distant from the cleavage site contribute toward high-affinity polypeptide binding by catalytic antibodies *(28)*. The proteolytic characteristics observed in the present study may be explained, therefore, as arising from formation of alternate ground-state complexes with different cleavage sites positioned in register with the chemically reactive subsite. When the antibody recognizes a conformational epitope in a large protein, the alternate cleavage sites must be spatially adjacent, but they can be distant in the linear sequence. In this model, production of polypeptide fragments with varying mass can be anticipated, as observed in the case of the gp120 substrate.

The present study is the first step toward isolating catalytic antibodies with antimicrobial therapeutic applications. Catalysts capable of gp120 cleavage at rates superior to the L chain described here could be isolated from the antibody repertoires of HIV-1 infected individuals or patients with autoimmune disease, both groups which are found to synthesize gp120-binding antibodies *(24,29)*. The specificity of gp120 cleavage can be enhanced by linking the catalytic V_L domain to a V_H domain that binds gp120, a suggestion supported by improvements in VIP binding affinity and catalytic efficiency observed in the case of an anti-VIP F_v construct containing a catalytic V_L domain linked to a VIP binding V_H domain *(30)*. Other issues that must be addressed concern the ability of the catalyst to recognize gp120 expressed on the surface of HIV-1, and the effect of serum, which might contain inhibitors of the reaction.

Acknowledgments

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Discussion

Sela: You have showed that the VIP sits inside a groove in the anti-VIP and then the VIP will be split at a remote site. Therefore I assume that the anti-VIP catalytic antibody does not proteolytically split other peptides. Is this correct? Then you showed us many different light chains that have other substrate specificities. Are all of them quite specific—for example, if you cleave prothrombin, is it only prothrombin and nothing else?

Paul: The specificity of these molecules is not absolute. This is partly because of the high sensitivity with which we detect the catalysis. The specificity ratio—that is, the cleavage rate of the specific substrate to unrelated peptides—is about 100 to 1.

Schoenfeld: I was very much impressed by the clinical aspects you mentioned. Is the incidence of the autoantibodies correlated with disease?

Paul: I think the statistical correlations have begun to emerge with the lupus patients. Of course, lupus patients have many other antibodies, including antiphospholipid antibodies. We are looking at a subset of lupus patients. I do not know whether the antibodies are involved in thrombosis, but I think it is attractive to test the hypotheses that these antibodies are directly involved in causing biological abnormalities. Regarding the thyroglobulin matter, in fact, some lupus patients do turn out to have silent thyroiditis. Yet these patients do not have the thyroglobulin-binding antibodies at high levels. We find the thyroglobulinases in some patients, but we have not looked at T3 and T4 levels. So these are really preliminary evaluations.

Tribbic: The specificity question poses a real challenge. Is it possible to map the exact epitope without initiating the catalytic response? Can you block the so-called "enzyme activity"?

Paul: We did some epitope mapping in which overlapping peptides of VIP were studied. We identified a seven residue epitope remote from the cleavage site. The seven residue peptide binds to the antibody and serves as a competitive inhibitor of the cleavage of full-length VIP.

Gabibov: Dr. Kaveri or Dr. Paul, I am excited to see this work. Like you, Dr. Nevinsky finds catalytic activity in healthy donors. My question is about the kinetics. Are the catalytic constants for thyroglobulin and the model small-substrate peptide different?

Kaveri: There is quite a large difference. The K_m for thyroglobulin is in the nanomolar range, the K_m for the short peptide is about 20 μM, and the V_{max} is nearly identical for the two substrates.

Gabibov: In this case, I don't understand your experiment with the inhibition. You inhibited cleavage of the model substrate by thyroglobulin, yet both substrates are cleaved equally. So what kind of inhibition do you get?

Paul: At nanomolar concentrations of thyroglobulin, there was a diminution in the rate of cleavage of Pro-Phe-Arg-MCA, which was held at micromolar concentrations. Good inhibition is seen because the thyroglobulin binds tightly.

Shoenfeld: Your lecture presented important evidence that there is a difference between pathogenic disease-associated antibodies and the natural autoantibodies in normals. The disease-associated are catalytic, and the nondisease associated are noncatalytic. Then you said that IVIG actually provides a supplement of catalytic antibodies. The question is: do you have

any idea why catalytic antibodies should be pathogenic? Is it possible that by catalyzing the cleavage of autoantigens, we actually expose some kind of an epitope in the immune system to generate antigen-specific T cells? Why are autoantibodies with catalytic activity generated?

Kaveri: We can only speculate. The catalytic process may cleave thyroglobulin and present the antigen fragments to the T cells, thus enhancing the autoimmune response.

Shoefeld: Why do we need the catalysts? We already have the disease; we already have the autoantibody; so why do we have to cleave the autoantigen to worsen the disease?

Kaveri: I cannot answer this question, but I can study the system further.

Koengten: I think we all agree that Darwin is right and Lamarck was wrong. We can only find in the immune response what is already there, so that we can select it. We can hypothesize that catalytic antibodies are always there, but they have just not been selected for. So when you start selection on an autoantigen, you get a side reaction that also generates catalytic antibodies, but the immune system itself cannot—by definition—select for catalysis. So the catalysis is just there by mere accident, but catalytic antibodies exist—otherwise you could never find tham.

Kohler: I would like to emphasize that IVIG effects can be associated with the differences in the idotypic reactivity and in alteration of the idiotypic profile in the patient. Perhaps you did some experiments in a mouse model by giving IVIG?

Kaveri: Yes, we obtained evidence in a mouse model for that proposition and published it in 1995.

Marchalonis: We found that rheumatoid arthritis patients have increased IgG autoantibodies to T-cell receptors compared to SLE or normals. Because we are always told that normals are not good controls, did you look at osteoarthritis patients as controls in your studies of rheumatoid arthritis patients?

Paul: Yes, osteoarthritis patients were studied. They did not show the decreased activity.