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NATURAL AUTOANTIBODIES CROSS-REACT WITH A PEPTIDE DERIVED FROM THE SECOND CONSERVED REGION OF HIV-1 ENVELOPE GLYCOPROTEIN gp120

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It was recently shown that peptide NTM (RSANFTDNAKTIIVQLNESV), corresponding to residues 280-299 in the second conserved domain of HIV-1 envelope glycoprotein gp120, has spectral and sequence similarity with human vasoactive intestinal peptide, VIP (Veljkovic et al., Biochem. Biophys. Res. Commun., 189, 705-710, 1992). We found that natural autoantibodies cross-reactive with this peptide can be detected in sera from HIV-negative asthma patients and healthy blood donors. The level of these antibodies is significantly higher in asthma patients than in healthy individuals, suggesting that these antibodies can in fact be at least partly identical to natural anti-VIP antibodies previously described (Paul et al., Biochem. Biophys. Res. Commun., 130, 479-483, 1985; Paul et al., Science, 244, 158-1162, 1989). Possible origin and role of these antibodies in AIDS pathogenesis and therapy are discussed.

The envelope glycoprotein gp120 of human immunodeficiency virus type 1 (HIV-1) is thought to play a central role in AIDS pathogenesis. The multifaceted nature of this principal HIV-1 antigenic determinant represents one of the main difficulties in developing methods for prevention and treatment of HIV infection. One intriguing property of HIV-1 gp120 is that it has an array of segments that are similar to crucial signal and effector molecules of the

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immune responses (2-7). Through this "molecular mimicry" HIV-1 gp120 may cross-react with the host's immune proteins that could in turn lead to the impairment of antigen presentation, disturbance of B cell function, disturbance of immune network dynamic and elimination of various immunoglobulins (8-11). As a consequence, the initially successful immune surveillance could become incapacitated and the persisting virus's chances to propagate would increase. A second consequence is that opportunistic pathogens, well-controlled in healthy individuals, can develop lethal infection in AIDS patients.

Recently, we have identified a region in the second conserved domain of HIV-1 gp120 (denoted as "peptide NTM") that has a high sequence and spectral similarity with human vasoactive intestinal peptide (VIP) (1). This peptide corresponds to residues 288-299 of gp120, using the numbering of the BH10 isolate. Subsequently, Neurath and co-workers reported that antibodies exist that recognize HIV-1 gp120 domain (a.a. 280-306), i.e., a domain that encompasses peptide NTM (12). It was also shown that asymptomatic carriers had significantly higher level of these antibodies than AIDS patients (12). Neurath and co-workers speculated that disappearance of these antibodies may represent an possible factor contributing to the development of AIDS. Since this region of HIV-1 gp120 is nonimmunogenic (13-15), the origin and function of these antibodies is presently unknown. The sequence and spectral similarity between peptide NTM and VIP leads us to the hypothesis that these antibodies could be natural anti-VIP autoantibodies (1). Anti-VIP antibodies have previously been reported in high concentration in sera from asthma patients (16) and in lower concentration in sera from normal healthy individuals (17,18). In order to check this hypothesis we have investigated sera from HIV-negative asthma patients and healthy blood donors for reactivity to peptide NTM derived from HIV-1 gp120. It has been demonstrated that sera from both groups contain antibodies cross-reactive with this peptide. Based on these results we offer some possible explanations concerning the nature and function of the NTM-crossreactive antibodies.

MATERIAL AND METHODS

Human sera. Sera have been collected from 14 patients (age between 20 and 40) with bronchial asthma (diagnosis made according to criteria defined by American Thotocic Society) and 9 healthy blood donors.

Peptide synthesis. The Solid Phase Peptide Synthesis of peptide NTM (RSANFTD NAKTIIVQLN), representing the consensus sequence derived from 28 HIV-1 isolate (SWISS-PROT, release 22) was made on a 9050 Milligen Peptide Synthesizer with Fmoc Chemistry and HONT/TNTU activation. The peptide was purified by high-performance liquid chromatography and concentration determined by quantitative amino acid analysis prior the use.

Determination of antibody binding to synthetic peptide as measured by ELISA. PVC microtitre plates (TITERTEC, Flow Lab.) were coated with 10 μg of synthetic peptide at concentration of 10 μg in 0.1M carbonate/bicarbonate buffer, pH 9.6 at 4° C overnight. Plates were blocked with 100 μl of 0.2% FCS in PBS containing 0.05% TWEEN at room temperature for 1h. Serial dilution of samples were made in triplicate in PBS/TWEEN (from 1/10 to 1/100000. Samples were added at volume of 100 μl and incubated overnight at 37°C. Goat and human IgG biotinylated whole antibody (AMARSHEM) was used as second antibody at dilution 1/1000 and incubated for 3h at 37°C. Streptavidin-biotinylated horseradish peroxidase complex (AMARSHEM) was added at concentration 1/1000 for 1h at 37°C. The plates were washed 4x between each step with PBS containing 0.05% TWEEN. Finally, the substrate o-phenylenediamine was added to wells and after to wells and after incubation of 15 min. at room temperature optical density (OD) was obtained at 492 nm in a BOEHRING ELMA PROCESSOR II. End-point titers to the synthetic peptide were considered to be the highest serum dilution which resulted in an OD greater than three times that obtained for a 1/100 dilution of control mouse serum.

RESULTS AND DISCUSSION

The binding curves of sera from asthma patients to peptide NTM are presented in Fig. 1. The results for healthy blood donors and asthma patients are summarized in Fig. 2. The results clearly show that all analyzed sera, both from asthma patients and normal individuals, contain antibodies that cross-reactive with peptide NTM. The titer of NTM-crossreactive antibodies in asthma patients is generally higher than in healthy blood donors (Fig. 2). The mean end-point titers for hyperimmune sera from asthma patients and normal sera were 1/10000 and 1/1000, respectively. It is interesting that similar relationship have been obtained for natural anti-VIP antibodies for these two groups (16-18). We also performed an anti-HIV ELISA test on all analyzed human sera and results were negative (results not shown). The above results together with the fact that peptides NTM, a domain homologous to VIP, is within the nonimmunogenic region of HIV-1 gp120, give strong support to the hypothesis that NTM-crossreactive antibodies detected in asyptomatic HIV-infected individuals could be natural anti-VIP autoantibodies.

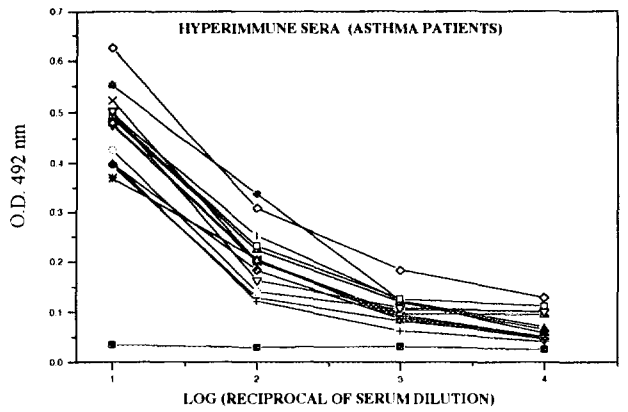

Fig. 1. The absorbance of sera from the fourteen asthma patients and control mouse serum (■) investigated for reactivity to the peptide NTM derived from HIV-1 gp120.

The above results may have interesting implications regarding the prevention of AIDS development. A synthetic VIP-derived peptide which also included the region of maximal homology with the peptide NTM have previously been shown to block HIV-1 gp120 binding and HIV infectivity and to serve as agonists of the CD4 receptor (19,20). It could thus be supposed that anti-VIP autoantibodies cross-reacting with peptide NTM homology region can prevent spread of HIV

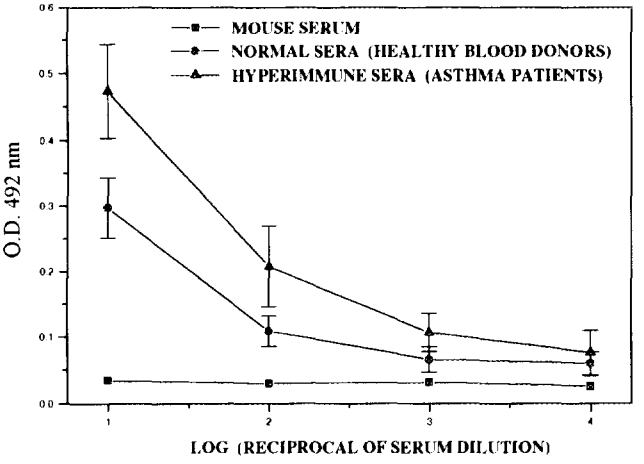

Fig. 2. The absorbance of sera from asthma patients (▲), healthy blood donors (●), and mouse (■) investigated for reactivity to the peptide NTM derived from HIV-1 gp120. Each point in curves for hyperimmune (▲) and normal (●) serum represents the mean value with brackets indicating ± SD for fourteen and nine individuals, respectively.

by blocking VIP-CD4-like interaction between HIV-1 gp120 and CD4 receptor. An alternative explanation can be based on the argument of Cohen and Cooke (21) who suggested that natural autoantibodies bind to self-mimicking epitopes (in this case epitope on gp120) and so prevent the initiation of damaging autoimmune response (frequently observed in AIDS patients). Thus, natural autoantibodies could act as a filter to ensure that only non-self epitopes impinge on the immune system and arouse a violent effector response. Deletion of such natural autoantibodies in HIV-infected persons could produce imbalance in immunoregulation and enhanced disease progression. It is noteworthy that, both of these explanations, if confirmed, would advocate the use of passive immunization either with hyperimmune sera containing high concentration of these antibodies or with human monoclonals (22). In addition, development of vaccine based on the peptide NTM and/or its derivatives may also become a promising alternative in the therapy or prevention of AIDS (22).

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