AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 8, Number 11, 1992
Mary Ann Liebert, Inc., Publishers

Reactivities of HIV-1 *gag*-Derived Peptides with Antibodies of HIV-1-Infected and Uninfected Humans

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ABSTRACT

A group of 41 peptides, each 24 amino acids long and overlapping with each other by 12 residues spanning the total *gag* open reading frame (orf) of HIV-1 (HTLV-III$_{BH}$ 10 isolate) were synthesized using F*moc* chemistry. The purified compounds were used in ELISA assays and tested for antibody reactivities in sera of human HIV-1-infected and noninfected individuals. Sera of HIV$^-$ humans showed reactivity against four defined regions, two in p17, one in p24, and one in p15. The values of these reactivities were elevated especially in serum samples of HIV$^-$ individuals showing cross-reaction with *gag* proteins on Western blot. Amino acid sequence comparison of HIV-1 *gag* proteins with those of human endogenous retroviruses (ERV K10, ERV 3) revealed significant similarities predominantly in the domains showing elevated antibody cross-reactions. The majority of sera from HIV-1$^+$ individuals showed strong reactivities to the cross-reactive regions and to various other peptide sequences, a sequential epitope recognized by all HIV-1$^+$ sera could, however, not be identified. The results suggest that human individuals may have immune reactions to endogenous retroviral protein sequences, which are enhanced by infections with HIV-1. Specific antibodies to HIV-1 *gag* proteins are probably mainly directed to tertiary structure defined epitopes formed by particle formation of the p24 monomers to the nucleocapsid.

INTRODUCTION

THE *gag* GENE PRODUCTS of human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS, are synthesized as a polyprotein precursor, p55, which is cleaved during particle maturation. The resulting products are p17, the matrix protein which is anchored into the viral or infected cell membrane by N-terminal myristic acid modification, p24 forming the nucleocapsid and p15, which is further processed into p9 and p6 and shows association with the viral RNA.[1-4] Antibodies directed against *gag* gene products appear early in HIV infection and are reported to decline with progression of the disease due to increasing antigenemia of p24, a phenomenon which may be used as prognostic marker for alteration of the patient's clinical status.[5-10] Since amino acid sequences encoded by the *gag* genes are highly conserved in various HIV-1 isolates[11] and also show a high degree of homology with HIV-2, antibodies directed especially to p24 may serve as a preferential diagnostic marker as well for early diagnosis as in follow-up studies of the infected patients during antiviral treatment. Tests using viral or recombinant p24 antigen on Western blots are, however, hampered by a significant degree of antibody-cross-reactions present in sera of not-infected human individuals. An enzyme-linked immunosorbent assay (ELISA) based on synthetic peptide antigens, where only conserved predominantly and specifically reacting compounds may be used should be able to avoid those effects representing an optimal test system.

Furthermore, due to their particle-forming capacity,[12,13] recombinant *gag* products may serve as potential vaccine candidate, which allow the inclusion of additional epitopes from other HIV-encoded proteins. A precondition for those developments is the exact immunological characterization of the individual domains present in *gag*-derived amino acid sequences. For this reason we synthesized 41 peptides spanning the whole *gag* polyprotein precursor, each about 24 residues in length overlapping with each other by 12 amino acids.

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The purified and characterized compounds were used in ELISA tests and assayed for reactivities with sera from HIV-1$^-$ humans, partly showing anti-p24 cross-reactions on Western blots and HIV-1$^+$ serum samples. The results are presented in this article.

MATERIAL AND METHODS

Peptide synthesis

Peptide synthesis was done using a 9050 PepSynthesizer (Milligen, Eschborn, FRG) using F*moc* (9-fluorenylmethyloxycarbonyl)-protected amino acids.[14] The first residue was coupled to polystyrene beads grafted with polyoxyethylene (Tenta-Gel, Rapp-Polymere, Tübingen, FRG). Double couple cycles were used at positions of the amino acid chain for the residues following proline or glycine and when equal or similar amino acid residues were clustered, in order to avoid incomplete coupling reactions. *t*-Butyl (Ser, Thr, Tyr), *t*-butylester (Asp, Glu), trityl (Cys, His, Gln, Asn), *t*-butyloxycarbonyl (Lys), and 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Arg) groups were used for side chain protection. F*moc*-protected amino acids were converted to hydroxybenzotriazol-activated esters with 1.5 mmol hydroxybenzotriazol and 1.2 mmol of diisopropylcarbodiimide per mmol of amino acid directly prior to the coupling procedure. The subsequent coupling reaction was performed in *N,N'*-dimethylformamide, coupling times of 10 minutes were used. F*moc* groups were removed by 20% piperidine, the completeness of this reaction was monitored by fluorometric measurement (all amino acids were purchased from Bachem AG, Heidelberg, Germany, solvents were from Merck AG, Darmstadt, Germany, chemicals from Aldrich, Steinheim, Germany). After synthesis, side–chain-protecting groups were removed by 4 h treatment in 50% trifluoroacetic acid in dichloromethane or, when Arg-residues were present in the peptide sequence, in 100% trifluoroacetic acid over night. Routine addition of 3% anisol and 3% phenol as scavengers, and 5% 2-mercaptoethanol was used in cases where Arg and Trp residues were combined in the peptide's sequence. Solvents were evaporated, the peptide suspended in 1–2 ml acetic acid and precipitated in an excess of ice-cold *t*-butylethylether, washed several times, and suspended in water. The peptide was lyophilized and purified by reverse-phase high-performance liquid chromatography (HPLC) using a C2/C18 copolymer column (PepS, Pharmacia, Freiburg, Germany) and a gradient of 0–70% acetonitrile in 0.1% trifluoroacetic acid. The peptide-containing fractions were lyophilized and characterized by amino acid sequencing (Applied Biosystems, Weiterstadt, Germany).

ELISA tests

Sera were selected according to their capacity to recognize viral or recombinant *gag* proteins on Western blots.[15] All sera were used in a dilution of 1:100 in phosphate-buffered saline (PBS), exceptions are indicated. Aliquots of 400 ng purified, characterized peptide per well were maintained overnight in 0.2 M sodium carbonate buffer, pH 9.5, in 96 well microtiter plates (Maxisorb, Nunc GmbH, Mainz, Germany). Free protein binding sites were saturated by 2 h incubation with gel solution (5 mg/ml, Sigma Chemicals, München, Germany). Before and after the addition of the serum dilutions the plates were washed several times with PBS/0.5% Tween-20. After incubation with human sera, plates were washed with PBS/0.5% Tween-20 and 0.9% NaCl. Rabbit anti-human IgG (Dako, Hamburg, Germany) was added in a dilution of 1:1000 in PBS. Staining was carried out in 0.1 M phosphate buffer, pH 6.0, containing 1 mg/ml *o*-phenylendiamine and 0.1% H_2O_2 for 10 minutes; the reaction was stopped with 1 M H_2SO_4 and the optical density was determined at 492 nm.

All sera were tested in double assays. For determination of specific reactivities, the mean values of reaction of second antibodies to the individual peptides without prior incubation with human sera were subtracted from the mean values obtained for the individual serum samples.

Competition assay

In the competition assay for p24, the antibody concentration of HIV-1$^+$ asymptomatic individuals was estimated by titration on Western blots. The serum concentration with the band of p24 (or p55) still visible on Western blots was used in the following assays. Western blot strips were prepared with HIV virus propagated on H9 cells and lysed in sodium dodecyl sulfate (SDS) solution. In the competition assays the serum was incubated without peptide for controls, with pools of p17-derived peptides (peptides 1–11), p15-derived peptides (peptides 30–41), and p24 (peptides 12–29). For each assay 4 μg of each peptide was used. After staining, densidometric evaluation of the Western blots was done (Elscript 400; Hirschmann, Unterhaching, Germany).

For the ELISA competition assay, first the optimal concentration of the recombinant p24 protein was estimated by titration. The recombinant protein (Mikrogen GmbH, Munich, FRG) comprises the whole sequence of p24, including 12 aa of p17 at the N-terminal part and 74 aa of p15 at the C-terminus. The proteins were coated at 20 ng/well and incubated with serum dilutions from 1:100 to 1:1600. The sera were preincubated with pooled peptides (peptides 11–35, 100 μg/assay), which spanned the whole sequence of recombinant protein p24.

RESULTS

Selected peptides

Forty-one peptides spanning the *gag* polyprotein precursor molecule (isolate HTLV-III/BH10;1) were synthesized. For length, peptides of 24 residues were selected, since these are able to form secondary structural motives and thus should allow not only the detection of antibodies directed to sequential epitopes but also of those directed to antigenic domains defined by structural properties to form α-helical, β-pleated sheet or β-turn protein regions. Occasionally peptides of 22 or 23 (peptides 1, 14, 15, 17) or 25 residues (peptides 27 and 30) were synthesized. This averted difficulties during synthesis and allowed us to obtain peptides with termini at the cleavage positions for p17/p24 and p24/p15 identical to those obtained by the procession of the polyprotein to the viral products p17, p24, and p15 in vivo. Each peptide had an overlap of 12 (or in some cases

```
      ←——p17
  1   MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL
      <---------- 1 ---------><--------- 3 ---------><---
                  <---------- 2 ----------><--------- 4 --

 51   LETSEGCRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA
      ------- 5 ---------> <---------- 7 ----------> <---
      --------><---------- 6 -----------> <--------- 8 ---

                                    p17 ——▶◀—— p24
 101  LDKIEEEQNK SKKKAQQAAA DTGHSSQVSQ NYPIVQNIQG QMVHQAISPR
      ------ 9 ----------> <---------- 11 ---------><-----
      ------->---------- 10 ----------><--------- 12 ----

 151  TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNTMLNT VGGHQAAMQM
      ----- 13 ---------> <--------- 15 ---------><------
      ------><--------- 14 ----------> <--------- 16 ------

 201  LKETINEEAA EWDRVHPVHA GPIAPGQMRE PRGSDIAGTT STLQEQIGWM
      -- 17 --------><--------- 19 ----------> <----------
      --->--------- 18 ----------><----------- 20 ------

 251  TNNPPIPVGE IYKRWIILGL NKIVRMYSPT SILDIRQGPK EPFRDYVDRF
      21 ----------><--------- 23 ----------><--------- 25
      ->-------- 22 ------------><--------- 24 ----------->

 301  YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTILKALGPA ATLEEMMTAC
      ------------> <---------- 27 ----------> <-----------
      <---------- 26 ----------> <---------- 28 ---------->

           p24 ——▶◀—— p15
 351  QGVGGPGHKA RVLAEAMSQV TNTATIMMQR GNFRNQRKMV KCFNCGKEGH
      29 ----------><---------- 31 ----------> <------------
       <---------- 30 ----------> <---------- 32 --------->

 401  TARNCRAPRK KGCWKCGKEG HQMKDCTERQ ANFLGKIWPS YKGRPGNFLQ
      33 --------> <--------- 35 ----------> <-------------
      <---------- 34 ----------> <--------- 36 ----------->

 451  SRPEPTAPPF LQSRPEPTAP PEESFRSGVE TTTPPQKQEP IDKELYPLTS
      37 --------> <---------- 39 ----------> <-------------
      <---------- 38 ----------> <---------- 40 ---------->

       p15 ——▶
 501  LRSLFGNDPS SQ
      41 -------->
```

FIG. 1. Amino acid sequence of HIV-1 *gag* proteins (isolate HIV-1/BH10;1) and location of the synthesized peptides. Locations of protease cleavage sites for processing of the precursor to p17, p24, and p15 are indicated.

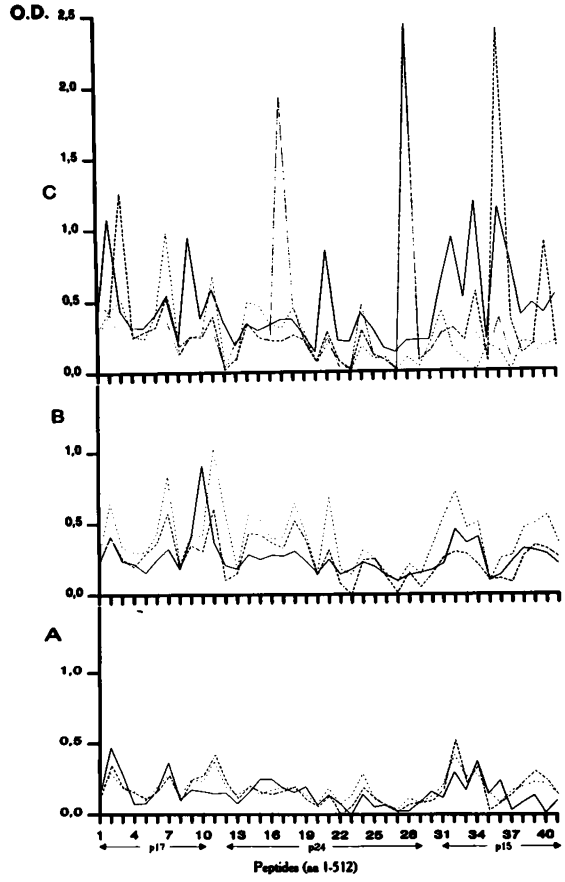

FIG. 2. Presentation and comparison of antibody reaction values to the synthesized *gag*-derived peptides in ELISA-assays. Values of 3 HIV-1 negative (A), 3 HIV-1$^-$ cross-reactive (B) and 4 HIV-1$^+$ sera (C) are shown.

11 or 13) residues with the neighboring ones. Sequence and location of the peptides on the *gag* proteins is shown in Figure 1.

Reactivity of HIV-1$^+$ sera

In order to test antibody reactivity to *gag*-derived peptides, sera of HIV$^+$ individuals were selected which showed a clear positive recognition of HIV-1 *gag* proteins on Western blot. Serum dilutions of 1:100 were used in order to avoid high background values due to unspecific binding to artificial structures. In general, reactivities of individual sera, when tested on *gag*-derived peptides, were relatively low showing values for optical density between 0.5 and 1.5. Higher reaction values were seen only occasionally and preferentially with peptides 2–3, 9–11, 24, and 31–34 representing the protein regions of amino acids 12–46, 97–145, 277–300, and 364–424 of the polyprotein, respectively. Further positive reactivities to other peptide antigens were observed in individual sera; these, however, could not be correlated with defined *gag* region (Fig. 2; Table 1). All sera showed positive and high reactions with a V3/gp120-derived consensus peptide[16] which was used as positive control. To exclude influences by different binding capacity of the individual peptides to polystyrene plates, various peptide concentrations (100, 200, 400, and 800 ng, 1 μg) and serum dilutions (1:10–1:400) were used, which did not lead to a significant and specific enhancement of reactivity; also, different coupling conditions on various ELISA plate systems had no positive influence according to serum reactivity. Since all peptides showed positive signals on Western blot we concluded that due to particle formation of p55 and p24, a process which is thought to be associated with major changes of the monomeric protein's structure, specific antibodies to *gag* proteins in HIV-1-infected individuals are directed mainly to structural motives. To test this assumption, we preincubated sera with peptide mixtures and determined the remaining reactivity to p55/p24 on Western blot and in ELISA assays using purified recombinant p24 as antigen. In none of the tests, any reduction of anti-*gag* reactivity could be observed (data not shown).

Reactivity of HIV-1$^-$ sera

When HIV$^-$ sera were tested on the individual peptides considerable reactivity with values for optical density up to 0.5 was observed in some serum samples despite the fact that all those sera showed negative reactivity to *gag* products on Western blot and in immunofluorescence (Fig. 2; Table 1). The peptides, which showed such positive reactivity were identical to those preferentially recognized by HIV-1 positive sera. In addition, weak reactivities were identified to peptides 6 and 7 (aa 60–95), a region where HIV-1 positive sera were rather inactive. Differences were only observed by quantitative means, the HIV-1 positive sera showing clearly elevated values. Those

TABLE 1. REACTION VALUES OF OPTICAL DENSITY WITH HIV-1 *gag* DERIVED PEPTIDES

	HIV⁻ non-cross-reactive 14 sera tested				*HIV⁻ cross-reactive 17 sera tested*				*HIV⁺ 48 sera tested*			
Peptides	*Weak*	*Middle*	*High*	*Significant reaction %*	*Weak*	*Middle*	*High*	*Significant reaction %*	*Weak*	*Middle*	*High*	*Significant reaction %*
1	1	—	—	—	1	1	—	6	24	6	2	17
2	9	3	—	21	10	3	4	41	12	16	16	66
3	2	—	—	—	7	1	—	6	21	3	4	14
4	—	—	—	—	1	—	—	—	4	1	1	4
5	—	—	—	—	2	—	—	—	1	1	—	2
6	2	—	—	—	4	1	—	6	9	1	—	2
7	4	—	—	—	4	2	1	18	8	4	—	8
8	—	—	—	—	2	—	—	—	4	—	—	—
9	7	1	—	7	7	5	—	29	6	6	4	21
10	2	—	—	—	4	3	—	18	9	1	—	2
11	5	—	—	—	6	2	2	24	8	6	1	14
12	—	—	—	—	3	1	1	12	13	1	—	2
13	—	—	—	—	2	—	—	—	1	—	—	—
14	1	—	—	—	4	2	—	12	6	2	—	4
15	1	—	—	—	3	1	—	6	4	2	—	4
16	1	—	—	—	3	2	—	12	6	2	—	4
17	1	—	—	—	4	1	1	12	5	1	1	2
18	1	—	—	—	5	2	1	18	9	4	—	8
19	2	—	—	—	4	2	—	12	2	1	—	2
20	1	—	—	—	1	—	—	—	2	—	—	—
21	3	—	—	—	4	4	—	24	7	2	—	4
22	—	—	—	—	2	—	—	—	1	—	—	—
23	—	—	—	—	3	—	—	—	6	—	—	—
24	5	—	—	—	4	1	—	6	9	3	2	10
25	—	—	—	—	2	1	—	6	1	—	—	—
26	—	—	—	—	2	—	—	—	2	—	—	—
27	—	—	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	2	—	—	—	1	1	5	12
29	—	—	—	—	1	—	—	—	1	1	—	2
30	—	—	—	—	3	1	—	6	1	1	—	2
31	—	—	—	—	6	2	—	12	11	2	—	4
32	9	—	1	7	6	6	2	47	12	20	5	55
33	3	5	—	36	6	2	5	35	17	9	22	65
34	5	5	—	36	4	7	4	64	5	14	12	56
35	—	—	—	—	1	1	—	6	4	5	1	12
36	4	3	—	21	2	5	1	35	13	18	9	57
37	1	—	—	—	1	1	—	6	6	2	1	6
38	—	—	—	—	5	1	—	6	2	1	1	4
39	2	—	—	—	7	1	—	6	8	1	—	2
40	—	—	—	—	5	—	—	—	5	5	3	16
41	—	—	—	—	—	—	—	—	1	2	1	6

Weak reaction: O.D. values 0.25–0.5; middle reaction: O.D. values 0.5–1.0; high reaction: O.D. values >1.0. Percentage of significant reactions for the respective sera groups are indicated and represent sera with high and middle reactivity.

reactivities could not be reduced by other methods for adsorption of peptide-free polystyrene plate surface with BSA (bovine serum albumin), milk powder, or gelatine. In addition, different procedures for washing after antibody exposition with high salt or different detergents had no effect and the observed reactivities were thus thought to be specific. HIV-1⁻ sera derived from children at the ages of 4 to 5 years were almost unreactive.

The positive antibody reactions to protein regions aa 12–46, 97–145, 277–300, 364–424 were enhanced when sera derived from healthy HIV-negative persons were used for testing, which showed positive reaction with *gag* products on Western blots. These sera showed values similar to those observed with HIV-1⁺ sera, especially for the peptides mentioned above. Occasionally, additional peptides were recognized; in some cases reactivities to one or two of the cross-reactive peptides were relatively low. Since sera of HIV⁻ individuals showed occasionally reactivities to *gag*-derived peptides similar to those of HIV⁺ patients it was not possible to define a specific cut-off value for the individual antigenic domains. Thus no peptide could be identified to react specifically with the majority of

TABLE 2. NUMBER OF IDENTICAL AND SIMILAR AMINO ACID RESIDUES IN THE INDIVIDUAL HIV-1 *gag*-DERIVED PEPTIDES WITH RESPECT TO HUMAN ENDOGENEOUS RETROVIRUS SEQUENCES ERV K10 AND ERV 3

	herv k10				*herv 3*			
	Identical		*Similar*		*Identical*		*Similar*	
Peptides	*Amount*	*%*	*Amount*	*%*	*Amount*	*%*	*Amount*	*%*
1	7	31	10	45	4	18	5	23
2	9	38	11	45	3	12	7	29
3	7	29	8	33	7	29	12	50
4	4	16	6	25	7	29	11	45
5	6	25	8	33	7	29	8	33
6	7	29	10	42				
7	6	25	9	38				
8	6	25	9	38				
9	7	29	12	50				
10	8	33	12	50				
11	9	38	13	54				
12	10	42	14	58				
13	8	33	9	38				
14	6	25	6	25				
15	2	8	4	16				
16	4	16	7	29				
17	5	21	9	38				
18	3	12	7	29				
19	4	16	7	29				
20	7	29	10	42				
21	9	38	10	42				
22	7	29	7	29				
23	8	33	10	42				
24	11	45	18	75				
25	8	33	12	50				
26	4	16	8	33				
27	9	38	13	54	9	38	12	50
28	10	42	14	58	10	42	11	45
29	12	50	15	62	5	21	6	25
30	10	42	13	54	6	25	9	38
31	5	21	8	33	10	42	14	58
32	9	38	11	45	7	29	10	42
33	10	42	13	54				
34	4	16	6	25				
35	6	25	7	29				
36	8	33	8	33				
37	4	16	5	21				
38	3	12	4	16				
39	5	21	5	21				
40	4	16	4	16				
41	2	8	2	8				

serum samples derived from HIV-infected persons: for regions with high reactivities high cross-reactions are also observed; specifically, reacting peptides in individual samples could not be correlated with a major antigenic domain, (Table 1, Fig. 2). Significant specific reaction values were estimated for reactivities showing values for optical density higher than 0.5 after correction for second antibody reactivities (see Material and Methods).

An amino acid sequence comparison of *gag* proteins, in dot plot analysis, derived from human endogenous retrovirus sequences (human ERV K10 and ERV3)[17,18] with HIV-1 *gag* proteins revealed stretches of homologous or similar sequences especially in the regions, where highest values of cross-reactivity with HIV^- sera were observed (data not shown). COMPARE and DOTPLOT or UWGCG computer program collections were used with similar amino acid residues included in the calculation.[19] Amino acid comparison of the cross-reactive *gag* peptides with aligned *gag* sequences of ERV K10 and ERV3 revealed, that up to 40 and 70% of the residues of the individual peptides were found to be identical or similar respectively (Table 2; Fig. 3).

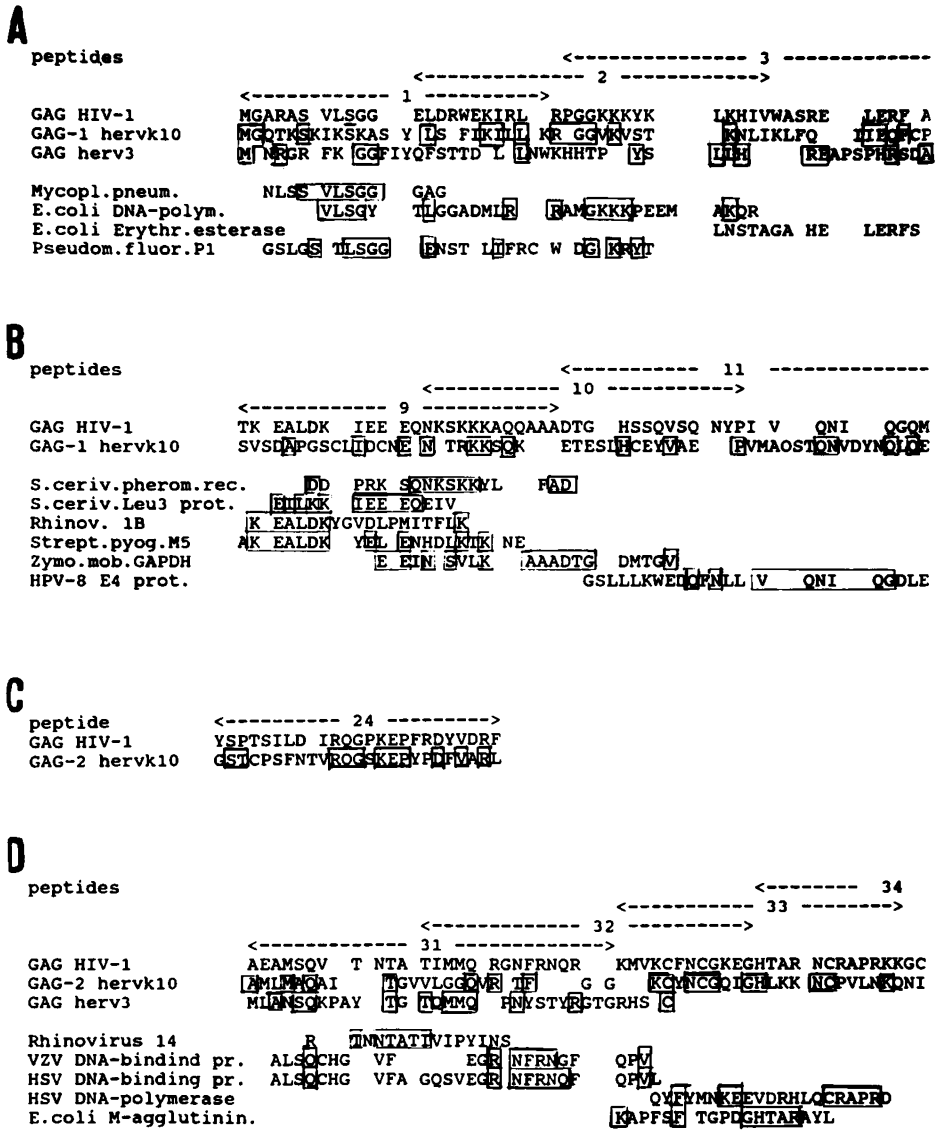

FIG. 3. Amino acid comparison of crossreactive regions. Equal amino acid residues are framed. HIV *gag* proteins are aligned with *gag*-1 and *gag*-2 proteins of human ERV K10 and human ERV 2 enogenous retrovirus sequences.[28,29] In addition, local similarities to bacterial and viral proteins of various origin are shown. (A) cross-reactive region 1 (aa 12-46, peptides 2, 3); (B) cross-reactive region 2 (aa 97–146, peptides 8, 9, 10); (C) cross-reactive region 3 (aa 277–300, peptide 24); (D) cross-reactive region 4 (aa 364–424, peptides 31, 32, 33, 34).

DISCUSSION

Testing HIV^+ human sera for antibody reactivity with purified peptides derived from *gag*-specific protein sequences in order to define antigenic regions revealed a highly problematic picture: Consistent reactivity was observed to protein regions, which were recognized positively also by HIV^- sera, reactions to other peptides were highly diverse in the individual serum samples. This leads to the conclusion, that a sequential epitope is not present in *gag* proteins of HIV and the majority of specific antibodies is directed to structural domains on p24/p55 formed by protein interaction of the polyprotein precursor molecules leading to particle formation. This is confirmed by the observation, that preincubation with peptide mixtures did not reduce reactions to p24 or p55. Other authors used different approaches to define antigenic domains in the p24-region of the *gag* polyprotein: Monoclonal antibodies to detergent-treated viral lysates or purified *gag* proteins were produced and were mapped to defined amino acid sequences using short overlapping peptides,[20–22] recombinant bacterial *gag* fragments,[23,24] or viral lysate[25] as antigen. These experiments led to the definition of antigenic epitopes in *gag* products, which, however show a rather broad variety in the individual papers, probably depending on the nature of the antigen used for immunization. Only one region (amino acids 210–225) is recognized rather constantly. The situation tested in those cases is, however, different from the *in vivo* infection in humans, since monomeric p24 not present during virus infection and replication has been used as

antigen. On those monomeric proteins antigenic regions which are secluded on the viral nucleocapsid by protein interactions may be exposed to the surface. Testing of the more natural situation by the use of human sera showed a high diversity in reaction when short peptides were used as antigens (9-mers),[21,22] which is similar to our finding where longer peptides (24-mers) were used, which allow the identification of secondary structure motives in addition to sequential epitopes. Regions recognized by mouse monoclonal antibodies also reacted with some human sera, but did not show preferential recognition: this finding may be explained by the highly diverse reactivities in the individual human serum samples.

The fact, that HIV^- sera show cross-reactivities especially with those protein regions that also show enhanced reactivities in $HIV\text{-}1^+$ serum samples implies that similar sequences have already been exposed to the immune system prior to HIV-infection. In other viral systems regions with similar amino acid sequences or similar epitopes leading to cross-reaction have been reported for cytomegalovirus.[26] In patients with autoimmune diseases like Sjögren's syndrome,[27,28] SLE and others cross-reactivities to *gag* proteins of HIV or other retroviruses were reported,[29,30] which may contribute to positive recognition of *gag*-derived peptides in individual sera.

Searching the protein sequences of NBRF and PIR banks revealed stretches of homologous amino acids present in polypeptides of bacterial or viral agents predominantly present in the regions responsible for high cross-reactivities described in this paper (Fig. 3). Since two of those regions, the aminoterminal part of p17 and the amino acid residues 364–424 of p15, comprise consensus sites for NH_2-terminal myristylation and nucleic acid binding in form of a potential Zinc finger region respectively, it has to be anticipated that sequentially and structurally similar domains are present in proteins of various infectious agents, which may contribute to the observed cross-reactions.

Amino acid sequence comparison of *gag* 1 and *gag* 2 proteins of human *erv* K10 and human *erv* 3 to HIV-1 *gag* revealed stretches of homologous or similar sequences especially in the region, where highest values of cross-reactivity with HIV-negative sera was observed.

Endogenous retrovirus are widespread in mammalians and are thought to make up at least 0.1 to 0.6% of the human DNA.[31] This feature, that DNA of normal uninfected cells contains gene sequences which are closely related to exogeneous infectious retroviruses, is unique among animal and human viruses. Their function is unknown, they possibly serve as a source of genetic variation, a reservoir for recombination, or may contribute to the origin of pathogenic retroviruses.[32] For the Gibbon ape leukemia viruses (GaLV) it could be shown that the DNA integrates into human cells.[33] Its sequence is indistinguishable from that of the fragments derived from the simian endogenous retrovirus SSAV (simian sarcoma-associated virus), where the *gag* and the *pol* regions contain sequences which are highly conserved in numerous retroviruses. GaLV is antigenically most closely related to a new world monkey virus, SSAV and less to the murine and feline C-type leukemia viruses.[34] In a similar way, antigenic relationship may be assumed between HIV and endogenous human retroviruses.

Only a few sequences present in human cellular DNA are known and in analogy with murine and simian systems, a large variety has to be assumed. All examples so far seem to be replication defective; however, some show transcriptional activity in individual tissues as human placenta and cell lines derived from human cancer or leukemia.[35–37]

The fact, that regions of HIV-1 *gag* products which exhibit distinct similarities to known sequences of human endogeneous retrovirus *gag* proteins show highest cross-reactivities in HIV^- sera point to the possibility, that those genes may be activated to protein synthesis during periods of postnatal life and lead to the production of antibodies or primed T-helper lymphocytes. This hypothesis can be supported by the finding of antibody reactions against capsid proteins of the simian viruses, SSAV and Mason Pfizer monkey retroviruses in HIV^- p24 crossreactive sera.[38] In those cases, where activated endogeneous *gag* sequences are similar to HIV *gag* proteins, infection with HIV may represent a "booster"-like effect resulting in the proliferation of antibody-producing B-cells, which can be stimulated by T-helper cells already primed for similar epitopes. Due to intracellular degradation of foreign antigen, and presentation of peptide sequences in combination with HLA class 2 proteins primarily sequential epitopes are recognized by those cross-reactions. Furthermore, experiments to vaccinate macaques with inactivated simian immunodeficiency virus (SIV) infected cells led to the discovery that protection was obtained by antibodies against certain cell components.[39] The nature of cellular components that led to protection in vivo is still unclear. Anti-*gag* cross-reactions, possibly based on immune reaction to endogenous retrovirus proteins, may contribute to the status of protection against SIV infection in unvaccinated animals.

In cases, when endogenous retroviral sequences are activated during embryonic stages of development, those sequences should be recognized as "self" proteins and immune reactivity against those and homologous proteins is avoided by education of premature T lymphocytes in the embryonic thymus.

Possibly both ways—activation of endogenous retrovirus sequences in postnatal life leading to a primed immune system with the production of specific antibodies, which may cross-react with similar domains on one side or activation during embryonic development as a result of clonal deletion of the respective T cells—may contribute to the highly diverse and complicated immune reaction found in humans to *gag* products during HIV infection.

ACKNOWLEDGMENTS

This work was supported by the Bundesministerium für Forschung und Technologie, grant FVP188, project 5. The authors thank Lutz Gürtler, Josef Eberle, and Friedrich Deinhardt, Max von Pettenkofer-Institut for HIV-positive and HIV-negative cross-reactive sera and many helpful discussions. Peptide sequencing was done by Reinhard Mentele, MPI for biochemistry.

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