HIV and the Sharpen Edge Between Protective and Pathogenic Immune Responses to the CD4 Self Antigen

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Abstract: The homeostasis of the immune function is severely impaired by HIV exposure, not only as an effect of the infection but also as a consequence of interactions of viral proteins with key elements of the immune system. In this context, in HIV infected individuals autoimmune responses to membrane CD4, the HIV receptor, are generally considered involved in the catastrophic loss of most part of the T helper lymphocytes. However, also in a portion of individuals naturally resistant to HIV-1 infection anti-CD4 antibodies appear, which are devoid of any harmful consequence and are associated to the lack of conventional signs of infection.

Here, we will focus on the differences of these two oppositely polarized outcomes, with particular reference to the fine specificities of these antibodies in HIV infected *versus* naturally resistant individuals and to the role of partially or totally CD4-gp120 complex-specific antibodies. We will review evidence supporting the notion that the fine tuning of the anti-self immune response to the HIV-1 receptor may contribute to determine whether viral exposure will bring to infection or, alternatively, to protective immunity.

The sharpen edge between harmful and protective self-reactivity appears as a key event in individuals naturally resistant to HIV infections with promising implications in the design innovative anti-HIV strategies.

Keywords: HIV, autoimmunity, CD4, natural resistance.

1. CONTROVERSIAL ASPECTS OF CD4 T CELL LOSS IN THE COURSE OF HIV-1 INFECTION

It is well established that depletion of CD4 T lymphocytes is the hallmark of the progression of HIV infection and, in the absence of antiviral treatment, the main contributor to the development of opportunistic infections and ultimately to the death of the majority of infected patients.

This is consistent with the fact that CD4 T lymphocytes physiologically orchestrate the whole immune response, by controlling the generation and the regulation of both the humoral and the cellular arms of acquired immunity against pathogens. In this framework, CD4 cell death in HIV infected individuals could be considered as the main pathogenic mechanism leading to immune suppression. However, there are several paradoxical observations which do not allow to consider this straightforward cause-effect relationships as the milestone of the HIV-triggered pathogenesis of the immune system. For instance, levels of immune activation, as assessed by proportions of CD38+ DR+ T cells and serum concentrations of beta-2 micro-globulin are closely correlated with disease progression and actually are more accurate disease predictors than CD4 cell counts or viral load. Indeed, HIV-1 infection leads to sustained activation of many key components of the immune system even in the very early stages and this is likely a fundamental mechanism for the ultimate collapse of immunity [1]. Thus, CD4 T cells do not appear as the mere victims of the infection, since they actively collaborate in the injury carried out against themselves and the immune system in general. The fact that this activity may be pathogenetically crucial in the destruction of immunity is also suggested by the model of sooty mangabeys, which are the natural host of SIV infection and, despite high levels of viral replication, experience neither immune activation nor disease progression [2]. Along this line, the majority of HIV-2–infected subjects who remain free from HIV-induced immune suppression, show negligible immune activation, whereas the latter in progressor subjects with HIV-2 is comparable to that seen in HIV-1 infection [3].

In this scenario, humoral and cellular self-reactivity have often been alleged to actively play a detrimental role, which could indeed explain how the relatively limited numbers of CD4 T cells actually infected by HIV-1 could nevertheless bring to the catastrophic loss of this cell type during disease progression.

In particular, autoimmunity could contribute to impair CD4 T cell functions in infected persons *via* reactivity to the CD4 molecule itself. Indeed, in a pilot study by Keiser *et al.* [4] and in our own experience (S. Burastero, personal observation) anti-CD4 antibodies were found to anticipate the appearance of antibodies to HIV-1 in exposed individuals, suggesting that they may indeed play a detrimental role since the first stages of infection.

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1876-8946/10

In this context, a further intriguing perspective in the interpretation of the conflicting existence of self-reactivity with anti-viral immunity was recently proposed by Verkoczy *et al.* [5]. These authors reported in a very elegant mouse model that rare neutralizing HIV-1 antibodies endowed with self-reactivity could activate central and peripheral immunologic tolerance. This model suggests that, among the mechanisms of natural protection from HIV infection, part of the humoral immune response could be harnessed by tolerance mechanisms themselves.

2. PATHOGENESIS OF ANTI-SELF REACTIVITY IN HIV INFECTED SUBJECTS: THE ROLE OF CELL DEATH AND APOPTOSIS

Several mechanisms have been described, which could support the development of autoimmunity in the course of HIV-1 infection. A specific role for the failure of regulatory T cell function was suggested by Oswald-Ritchter *at al.* [6] whereas Rawson *et al.* [7] focused on the increased tendency of CD4 T lymphocytes from infected individuals to undergo activation-induced death or apoptosis and demonstrated the subsequent presentation of remarkable amounts of self-epitopes. These Authors found that apoptotic events were capable to break tolerance and trigger cytotoxic T cell-mediated autoreactivity towards several autoantigens, such as myosin, vimentin and actin [7]. Apoptosis is an ordered state of cell death in which the structural components of the cell are carefully disassembled by the activity of a unique set of proteolytic enzymes, notably members of the caspase family. The self-proteins broken down by caspases in a multitude of apoptotic cells can prime cytotoxic T lymphocytes (CTLs) through subsequent proteasomal digestion and cross-presentation. Thus, the massive death and destruction of lymphocytes due to the cytopathic action of HIV-1 could force the breaking of tolerance to self-peptides and permits the generation of autoreactive CTLs responding to the cleavage products of apoptotic cells.

Naturally SIV-infected species which do not display any detectable signs of either immune deficiency or autoimmunity represent a non-human primate model of HIV infection which might shed light on the pathogenesis of autoimmune phenomena in infected humans [8]. The cytopathic effect of viral loads [9], anti-viral humoral responses [10] and a variety of cell signalling pathways [11] were ruled out. In the same time, several other mechanisms were identified, including innocent bystander killing by apoptosis of CD4+ T cells, the anomalous regulation of the cell cycle, and the fact that distinct host proteins can get incorporated by the virions as they are assembled and either bud out of CD4+ T cells or exit the infected cells [8]. However, these observations do not account for the whole story, since in the acute infection of Sooty Mongabey, although they loose the great majority of $CD4^+$ T cells in the Gut Associated Lymphoid Tissue (GALT), they go on to tolerate lifelong high levels of viral replication without any evidence of immunodeficiency [12].

3. PATHOGENESIS OF ANTI-SELF REACTIVITY IN HIV INFECTED SUBJECTS: UNVEILING OF CRYPTIC EPITOPES BY ALTERED ANTIGEN PROCESSING AND THE INTER-MOLECULAR HELP MECHANISM

An autoimmune cytotoxic T-cell response to the CD4 molecule was described in HIV-1 positive patients [13, 14] whose pathogenesis was proposed to depend on the unveiling of cryptic epitopes following internalization of CD4 in complex with gp120 [15, 16]. A further *in vivo* proof of principle of the importance of this mechanism was provided by pilot experiments of T-cell vaccination against anti-CD4 autoimmunity in a small sample of HIV-infected patients [17], which provided a significant improvement of anti-HIV immunity.

Several intracellular interactions of newly synthesized CD4 molecules with various HIV proteins were described, which may induce the generation of various self-epitopes, ignored by tolerance mechanisms in the absence of HIV molecules. For instance, the formation of Env (gp160)-CD4 complexes in the ER can lead to their retention *via* binding to Vpu, which re-direct them to degradation [18-21]. Similarly, Nef interaction with the cytoplasmic tail of membrane CD4 was reported to prompt its transport to degradation organelles [22].

In conclusion, autoimmunity to CD4 in HIV-1 infected patients is supported by several mechanisms concurring to the generation of cryptic epitopes and to the activation of T cells not previously deleted by central tolerance during the maturation of the T cell repertoire.

As an alternative, not mutually exclusive hypothesis for the generation of anti-CD4 antibodies, the so-called "inter-molecular help" phenomenon was proposed. This mechanism implies that gp120-specific T cells provide help for antibody production to CD4-specific B cells recognizing B-cell epitopes on a gp120-CD4 complex [23]. Although the *in vivo* relevance of this specific occurrence has never been established, it appears as a reasonable mechanism, reminiscent of the redirected antigen-presentation, which follows presentation of antigens complexed with antibodies with different fine specificities [24, 25].

4. ANTI-CD4 ANTIBODIES CAN BE NON-IMMUNE SUPPRESSIVE: LESSONS FROM CLINICAL TRIALS

As expected from basic immunology notions, anti-CD4 antibodies have long been proposed as immune suppressors for the treatment of human autoimmune diseases [26]. However, divergent effects were observed in clinical trials, particularly in the treatment of rheumatoid arthritis, where a promising initial efficacy in open anti-CD4 studies [27, 28] was followed by subsequent discouraging results in double-blind clinical trials (reviewed in [29]). Later on, a revitalization of the anti-CD4 treatment notion with new, humanized anti-CD4 mAbs [30] was observed. The basis for these inconsistencies relies on the complexity of anti-CD4 influence on the immune system. Indeed, in early studies, anti-CD4 mAbs were found capable to induce either cell depletion [31] or functional inactivation of T cells [32, 33], although activation of T-cell functions was also reported[34]. Moreover, it has long been known that anti-CD4 monoclonals are immune suppressive or tolerogenic depending on the circumstances of their administration [35-37]. Along this line, it is generally recognized that non-depleting monoclonal may be relatively more effective in tolerance induction, for instance in the treatment of rheumatoid arthritis [27], psoriasis [38], systemic lupus erythematosus [39] and multiple sclerosis [40]. However, only inconclusive and temporary symptom

relief was accomplished in preliminary open studies. The fine epitope specificity of anti-CD4 antibodies may play a role in this context, as demonstrated in a model of rat adjuvant arthritis, where the developmental pattern of the disease differed substantially between three distinct monoclonals, two of them preventing, the third one accelerating the development of the disease [41]. The effect of each reagent on the signaling activated by CD4 *via* the $p56_{lck}$ interacting cytoplasmic tail is supposedly implicated in these differences.

In this context, the usage of human derivatives of mouse monoclonals allowed on one side reduce the generation of xenogeneic reactivity inherent to rodent monoclonals, on the other to modulate the induction of effector mechanisms. In engineered derivatives, the isotype used (*e.g.*, IgG1 versus IgG4) has implication on complement fixation capability and on the binding to Fc receptors bearing cells, whereas variation in the number of binding sites (*e.g.*, single chain constructs, Fc fragments, *etc.*) implies modification of functional effects of the original reagent. Recently, a fully human anti-CD4 monoclonal antibody (HuMax-CD4) was tested in a multicenter, double blind, placebo-controlled, randomized clinical trial on moderate to severe psoriasis patients, showing decreases in the psoriasis skin score, although this failed to reach statistical significance [42].

Further complexity to be considered when using *in vivo* CD4-interacting reagents derives from the fact that two sets of NFAT binding sites were identified in the HIV-1 long terminal repeat (LTR) promoter, and CD4 engagement can result on the $p56_{lck}$ kinase dependent activation of both cellular transcription factors and HIV-1 LTR [43]. Thus, a signaling trigger *via* CD4 can activate both the endogenous and the retroviral NFAT family of transcription factors, simultaneously inducing both T cell activation and increased transcription of the viral genome [44]. This phenomenon might explain the observation that HIV-1-positive transplant recipients reduced viral burden during treatment with cyclosporin A (CsA) [45], a potent inhibitor of these transcription factors. Moreover, CD4 dimerization occurs when CD4 membrane cell density exceeds 10^5 per cells, a phenomenons which involves D4-D4 domain interactions and could *per se* triggers auto-phosphorylation and T cell activation [46].

In conclusion, the effect of anti-CD4 in human therapy is far from being the expected straightforward immune suppression and is influenced by so different factors as subtle differences in epitope specificity, isotype and number of binding sites.

Recently, one anti-CD4 antibody (ibalizumab) which does not induce any relevant immune suppressive effect *in vitro* or *in vivo* was tested in phase II clinical trials, in the form of human IgG4 derivative. Ibalizumab acted as promising entry inhibitor, which was capable to block HIV-1 infection without inducing any immunologically relevant side-effect [47, 48]. Notably, this molecule recognizes an epitope mapping to the second CD4 domain and does not significantly interferes with HIV-1 docking on the cell membrane. The anti-viral activity of ibalizumab is explained as a consequence of the interference on conformational changes taking place on the cellular HIV-1 receptor at the post-binding level [49].

5. ANTIBODIES TO THE CD4-gp120 COMPLEX

A sequence of pre-ordered conformational changes takes place orderly following CD4-gp120 interaction on both moieties of the complex. These modifications are vital to the viral cycle events, in that they do not only allow gp120 interaction with coreceptor but also prompt membrane fusion and viral entry into the cells. From the immune system perspective, this conformational flexibility generates a series of transitorily expressed antigenic determinants, which dynamically re-design the epitopic make up of interacting moieties and ends up with several new determinants becoming accessible at the molecular surfaces of the antigens.

The binding of gp120 to CD4 involves a well-defined site within the first Ig-like domain of CD4 (CD4 D1) [50] at the level of the Phe43 CD4 residue [51]. The latter is docking into a conserved hydrophobic pocket of gp120 which appears as a discontinuous region at the interface between its inner and outer domains [52]. Notably, the lateral face of this same D1 CD4 domain is implicated in MHC-class II interaction, an event physiologically providing an activation signal which is involved in several physiological and pathological T lymphocyte functions [53]. This molecular location makes it possible for D1-CD4 domains specific antibodies to interfere with physiological immune functions.

CD4 induced (CD4i) determinants are those epitopes, which are exposed on the gp120 molecule after binding to the cellular receptor. All known CD4i antibodies recognize a common, conserved gp120 element overlapping the binding site for the CCR5 chemokine receptor [54]. We characterized a gp120 neutralization epitope, recognized by the D19 murine monoclonal antibody, which is differentially accessible in the native HIV-1 Env according to its coreceptor specificity [55]. In CCR5-restricted (R5) isolates, the D19 epitope was invariably cryptic, although it could be exposed by the addition of soluble CD4; epitope masking was dependent on the native oligomeric structure of Env, since it was not observed with the corresponding monomeric gp120 molecules. By contrast, in CXCR4-using strains, the D19 epitope was constitutively accessible. In accordance with these results, R5 isolates were resistant to neutralization by D19, becoming sensitive only upon addition of sCD4, whereas CXCR4-using isolates were neutralized regardless of the presence of sCD4 [55]. Taken together, these observations can be deciphered in evolutionary term by saying that CD4-induced changes in gp120 conformation are functionally crucial for HIV-1 entry, and illustrates a viral strategy for sequestering the chemokine receptor-binding region of gp120 away from the attacks of the humoral immune response [56].

Along this line, similar observations can be reciprocally applied to the CD4 receptor. Indeed, complex specific epitopes on the CD4 moiety have been identified with partially or totally complex-specific monoclonals antibodies, which do not interfere with the CD4-Env complex formation, such as CG10 [57] and "antibody 55" [58], both mapping to the second Ig-like CD4 domain. We recently generated an anti-D2 CD4 monoclonal antibody (DB-81)[59, 60] not interfering with gp120 binding and with a binding affinity around 700 times higher for CD4 complexed to gp120, as compared to CD4 (Burastero SE *et al.*, in preparation). Notably, CG10 is weakly interfering with membrane fusion and HIV replication [57], whereas DB-81 reacts with both membrane-bound

and solid-phase coated recombinant CD4. Moreover, DB-81 displays a broad spectrum of neutralization, suggesting that little differences in the fine specificity may imply relevant impact on the capability to interfere with the chain of events which follows viral docking on the cell membrane.

6. NATURALLY PRODUCED ANTI-CD4 ANTIBODIES, SUSCEPTIBILITY TO HIV INFECTION AND SPECIFICITY TO THE SECOND EXTRACELLULAR CD4 DOMAIN

Autoimmune responses towards CD4 detected in HIV-1 infected individuals have long been known to bind to solid-phase recombinant-, but not membrane bound-CD4 expressed on T lymphocytes or cell lines [61, 62] (Burastero, personal observations). Notably, these antibodies are not targeting the virus-binding domain [63] but preferentially recognize epitopes masked by the physiological dimerization of CD4 on the cell membrane. This observation suggests that they are derived from an extensive processing of the self antigen, which made hidden epitopes "emerge" on antigen presenting cells.

Consistently with these findings, extensive epitope scanning mapped CD4-specific T cells in HIV-1 positive individuals to any of the four CD4 domains [64]. In contrast, the little proportion of CD4-reacting IgG from healthy individuals are specific for epitopes of extracellular CD4 domains.

In agreement with these observation, Denisova *et al.* [46] reported that immunization of hu-CD4 C57Black/6J mice with HIV-1 gp120(451) complexed with its receptor protein produced, in the tolerogenic hu-CD4 transgenic background used to mimic the human situation, two anti-CD4 monoclonal antibodies, designated T6 and T9, mapping to the D3-D4 domains and recognizing soluble but not membrane associated CD4. These antibodies were capable to compete with anti-CD4 antibodies detected in HIV-1 infected people.

In contrast to this situation, a surprise came from the study of individuals who, despite repeated expusure to HIV, do not develop infection (ESN, exposed uninfected) because of natural resistance, a multifaceted phenomenon which still awaits to be fully clarified. Among other immunological and non- immunological peculiarities, a portion of ESN individuals display autoimmune traits that can be referred to HIV exposure, including the distinctive reactivity towards the CD4 molecule [65]. We formally demonstrated that an inter-molecular help mechanism could explain the breaking of tolerance and the production of IgG antibodies to CD4 [66]. Notably, also newborn babies from seropositive mothers were found to display this autoimmune trait, characterized by an initial increase of antibody titers followed by anti-CD4 decrease and viral clearance [67]. These antibodies are likely part of a more general anti-cell immunity, including specificities to CCR5, the HIV- coreceptor [68].

Anti-CD4 antibodies in ESN subjects bind to both membrane and soluble CD4 and have syncytium inhibiting activity [65]. Along this line, the presence of distinct fine specificities in the repertoire of anti-CD4 antibodies in ESN subjects as compared to HIV-1 infected people was later confirmed in large cohorts of individuals of ethnically different origin who were investigated for anti-CD4 and anti-CD4-gp120 complex specific IgG antibodies [68]. Moreover, a clear-cut prevalence of complex-specific antibodies in ESN versus HIV-infected subjects was demonstrated [68]. The active role of these antibodies in HIV resistance was suggested by the segregation of these peculiar fine specificities not only in naturally resistant individuals but also in long-term non-progressor patients [69].

Anti-CD4 antibodies in ESN subjects are one among several signs of unconventional immunity, which were described in HIV-1 resistant individuals [70]. Preliminary evidence from ESN individuals (Burastero *et al.*, personal observation) suggest that specificity to the second domain of CD4, with particular reference to strictly conformation-dependent epitopes, and including those, which are preferentially expressed after gp120 binding may be associated with a non-harmful and potentially protective humoral anti-HIV-1 autoimmune response. Further systematic studies are needed to characterize anti-CD4 antibodies fine specificities in healthy subjects, with or without HIV- exposure, and to determine their HIV-1 inhibitory capability. Available evidence suggesting a protective role of anti-CD4 antibodies is summarized in Table **1**.

Molecular structure analysis of free versus bound CD4 may be helpful in shedding light on the above reported observations. Notably, the alignment of the corresponding structures extracted from available database results in a virtually complete overlap of their backbones (Root Mean Square Distance <0.7 Å) [71]. However, when C-alpha atoms B-factors are considered as a measure of local backbone mobility [72], the first CD4 domain does not display significant variations of local backbone mobility, with the expected exception of the region in close contact with the surface of gp120. In contrast, the second domain displays large variations, suggesting that the D2 CD4 domain, despite the fact that it is not directly involved in binding, significantly reduces its local flexibility [71]. A representation of areas which are involved in gp120 interaction (first CD4 domain) and which are specifically protruding, according to molecular dynamic analysis, after interaction with gp120 (second CD4 domain) are shown in Fig. (**1**).

Table 1. Protective Role of Anti-CD4 Antibodies

1)	Anti-CD4 antibodies have been identified in individuals who are sexually exposed to HIV, yet they do not subsequently develop either anti-gp120 antibodies or clinical signs of infections (Esposed SeroNegative, ESN) [65, 68].
2)	Anti-CD4 antibodies have been identified in newborn from seropositive mothers, whose titres are increasing in the first months after birth, and subsequently decreasing in association with viral clearance [67].
3)	Anti-CD4 antibodies have been characterized in Long-Term non Progressor HIV-infected individuals [69].
4)	Anti-CD4 antibodies isolated *ex vivo* from Exposed SeroNegative individuals display *in vitro* inhibition of HIV-mediated fusion of target cells [65].

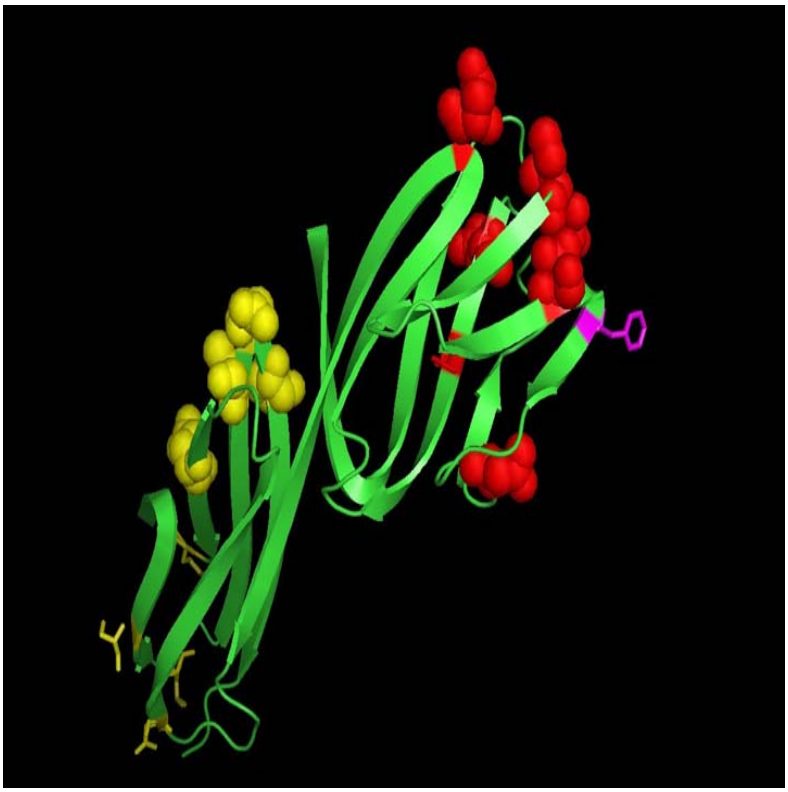

Fig. (1). First (top-right) and second (left-bottom) domains of CD4 structure (from accession number 3CD4). The gp120 binding area of CD4 maps to the first CD4 domain (in violet the critical contact residue Phe43 is depicted, surrounded by other contact residues in red, representing in space-fill mode the area involved in coreceptor binding). Residues in yellow correspond to the areas containing epitopes prevalently protruding, according to molecular dynamic analysis, after interaction with gp120. These areas are preferentially recognized by IgG autoantibodies to CD4 from exposed seronegative (ESN) individuals and by monoclonal antibody DB-81 (yellow residues in space fill mode) which interfere with HIV entry and membrane fusion without preventing CD4-gp120 interaction.

Thus, it appears the conformation of the membrane molecule serving as viral receptor has a certain degree of flexibility of solvent-exposed determinants, which is decreased following ligand binding. This decrease occurs not only, as expected, in the direct proximity of the binding site, but also in extended portions of the second CD4 domain.

This phenomenon may explain the potential inhibiting capability on viral entry by strictly conformational antibodies, or derivatives thereof, specific to such protruding, "stiffer" epitopes. Moreover, since such a locally rigid antigenic make up is by definition transient, and the corresponding set of epitope is limited, it may be in principle associated with an overall lower immunogenicity. However, available data on anti-CD4 antibodies in ESN demonstrate that a proportion of individuals can indeed spontaneously produce antibodies with these fine specificities. These may pre-exist as the results of previous exposure to different (non HIV-related) antigenic stimuli, they may be natural antibodies with relatively low affinity, and/or may be subjected to affinity maturation following HIV-1 exposure. The characteristics of antibodies binding to the second Ig-like extra-cellular CD4 domain are summarized in Table **2**.

CONCLUSIONS

Individuals naturally resistant to HIV-1 infection represent an experiment of nature whose study has potential im-

Table 2. Characteristics of Antibodies Binding to the Second Ig-Like Extra-Cellular CD4 Domain

1)	The second immunoglobulin-like domain of CD4, which is not involved in CD4-gp120 interaction neither in interaction with MHC class II, is prevalently targeted by anti-CD4 antibodies from ESN subjects, whereas these autoantibodies in HIV-infected patients recognized determinants mapping to the four (intra-cellular plus extra-cellular) CD4 domains [64].
2)	Monoclonal antibodies whose fine specificities is restricted to the second Ig-like extracellular domain of CD4 are not immune suppressive, yet they are capable to interfere with the chain of conformational modifications which follow viral docking and subsequent entry into CD4-expressing target cells [47].
3)	Anti-CD4 antibodies which preferentially recognize CD4 epitopes exposed following gp120 interaction map to the second Ig-like extra-cellular CD4 domain [71]. This is in agreement with molecular dynamic testing comparing free *versus* gp120-bound CD4 structure. This analysis assigns to the D2 domain relatively higher levels of exposure of determinants protruding from molecular surface after formation of the CD4-gp120 complex [71].

plication for the design of alternative immunological therapies of HIV-1 infection. Anti-CD4 antibodies are not subjected to the immune evasion, which characterize Env-specific immunity, nor to the generation of resistance, which impairs the efficacy of antiretroviral therapy with non-entry inhibitors. Thus, the possibility to elicit non-immune suppressive, protective anti-CD4 immune responses or, alternatively, to use monoclonal antibodies or derivatives thereof, which will reproduce this activity may dramatically improve therapeutic options for HIV-1 treatment in the next few years.

A long-standing effort has been attempted to target conformation-specific epitopes, as a strategy to overcome the failure of conventional vaccination approaches to prevent HIV-1 infection [73-75]. The data we review here suggest that the fine characterization of crucial epitopes recognized by antibodies from ESN subjects will allow to increase the chances to successfully implement this strategy.

ABBREVIATION

ESN = Exposed Sero-Negative

ACKNOWLEDGEMENTS

SB was funded by Istituto Superiore di Sanità, Rome, AIDS Program.

The funding body had no influence in the study design, collection, analysis and interpretation of data, writing of the manuscript and in the decision to submit the manuscript for publication.

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Received: November 02, 2009 Revised: February 12, 2010 Accepted: February 15, 2010