

Is Autoimmunity to CCR5 an Escape from the Intrusive HIV?

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Abstract: Albeit strictly controlled in immune function and in ontogenesis, autoimmunity may develop upon inflammatory response to pathogens, with harmful consequences to host. Findings of autoimmune-like responses, observed in HIV-infected patients, might play a key role in modulating the natural history of the infection and/or could even confer protection to HIV. This paper will focus on a special class of auto-antibodies observed in HIV exposure, anti-CCR5 IgG and IgA and on their protective potential.

Keywords: CCR5, HIV, antibody, autoimmunity, natural immunity.

1. INTRODUCTION

Microbial infections usually determine a complex cascade of events in the host, aimed at producing protective responses and at clearing pathogens promptly, with limited or no harm for the host. Some events, as the generation of pro-inflammatory stimuli and the local recruitment of immune cells, are beneficial in the infection environment; these events can nevertheless turn harmful when they become uncontrolled and determine undesired consequences for the host. Microorganisms and of course HIV have successfully learnt how to exploit host defences at their own advantage, for example by turning signalling molecules off or by mimicking host antigens to conceal themselves from the immune surveillance.

Pathogen blocking or neutralization mediated by host antibodies is often effective in preventing infection and spread of viruses. In HIV infection, a huge antibody production is usually observed after primary infection; however, most of these antibodies are generated in response to the state of general inflammation and immune dysfunction and fail in protecting the host. Neutralizing antibodies do not usually develop in early phases of HIV infection; most importantly, only a percentage of infected individuals succeed in generating specific antibodies blocking the virus.

HIV antigens, provided to the host in vaccine formulations, have almost completely failed in inducing protective - or at least therapeutic - responses. Indeed, during the natural history of HIV infection, some paradoxical responses, such as the induction of anti-self antibodies, were observed [1, 2]. Initially considered as a reflex of the virus-induced hyperstimulation, the auto-antibodies have been reconsidered over time as host's attempts to counteract HIV infection. One of these intriguing findings deals with CCR5, the major coreceptor that mediates HIV primary infection and spread from human mucosa. The biological properties of anti-CCR5 antibodies and their induction will be introduced and discussed here.

2. ALLO- AND AUTO-IMMUNITY

Anti-cell responses are uncommon but possible in healthy individuals, since auto-reactive antibodies are usually found in blood specimens from healthy donors or in commercial pools of human sera [3-6]. Natural allo-immunization is usually observed when host cells enter in close contact with cells and tissues carrying different HLA haplotypes, for example in pregnancy, blood transfusion or organ transplantation; it was also used in clinical prevention of abortion [7, 8]. CD4+ T cells from allo-immunized subjects displayed a reduced susceptibility to *in vitro* infection with laboratory and primary HIV strains [9]. This effect was explained by the presence of HLA-I and -II molecules on HIV surface [10, 11]. Auto-antibodies generated in allo-immunized, HLA-discordant couples, blocked HIV isolates *in vitro*, a finding also confirmed by experimental immunizations [12, 13]. Unprotected sex (homo- and hetero-sexual) was shown to induce stronger allo-immune responses in both CD4+ and CD8+ T cells than the condom-protected intercourses; the increase of anti-HIV responses in PBMC was also observed [14]. Some couples enrolled in the study also showed a higher number of activated CD4+CD25+ T regulatory cells, a finding suggesting that unprotected sex elicited tolerization to HLA antigens from the partner. Tolerization of CD8+ T cells affected a significantly higher number of homosexual than heterosexual couples; this fact, possibly explained by anatomical and immunological differences of rectal vs genital mucosa, underlines the key role of local factors in the generation of genital as well as of systemic immunity to HIV [14].

Auto-antibodies were observed and significantly associated with late stages of HIV infection, suggesting the existence of an autoimmune-like dysfunction that might account for progressive lymphopenia, thrombocytopenia, hypergammaglobulinemia and other clinical features observed in the disease [15-19]. Nevertheless, the presence of anti-cell antibodies in HIV-exposed uninfected people strengthened the hypothesis of a response involved in the development of natural resistance to HIV [20-22].

Other hypotheses about autoimmunity observed in HIV patients considered the molecular similarity among some

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epitopes in the Env proteins and various host antigens, such as IL-2, HLA-I and -II, complement factors C1q and CFH, integrin beta-3 GPIIIa, the astrocyte isoform of alpha-actinin [23-29]. Of note, some of HIV host-mimicking epitopes, such as those corresponding to IL-2, are immunodominant; consequently, “*the more the immune system fights against the virus, the more it fights against itself*” [26, 27].

Anti-cell antibodies were first described in macaques, as spontaneous responses; subsequently, anti-HLA and anti-lymphocytes antibodies were found in sera from SIV-infected macaques and sooty mangabey monkeys [30]. Studies of allo-vaccination in monkeys confirmed that repeated contacts with allo-antigens from seminal fluid or fetuses increased T cell count, chemokine secretion and also induced anti-CCR5 antibodies, all mechanisms resulting in natural resistance to SIV infection *in vitro* [31]. Monkeys immunized with human uninfected cells were protected from the challenge with HIV strains grown in human cells; protection was due to the xenogenic response against human proteins, such as the HLA molecules, that were exposed at the surface of viral particles [32]. Anti-CCR5 monoclonal antibodies blocked HIV infection *in vitro* and protected monkeys from challenge with SIV strains, once passively administered [9, 33, 34].

3. THE GENERATION OF AUTOANTIBODIES

The immune system is a complex defensive army and each of its branches is endowed with different mechanisms and timing for response to pathogens; in addition, each branch can successfully discriminate “self” from “non-self” elements. Complexity should provide proper and prompt protection in a wide spectrum of situations without endanger host safety. In other words, the almost infinite repertoire of potential immune responses has some limitations. Many pathogens, especially the stealth ones, are used to exploit system faults and “grey areas” to infiltrate and conceal themselves from the host immunity; their efforts sometime succeed in establishing acute or chronic colonization, latency and escape from the host defence. Two excellent players in this spy-story are the Herpesviruses and the Endogenous Retroviruses (ERVs). These viruses have achieved the widest spread in mammals, primates and human populations since million years, usually without causing severe or life-threatening damage to their hosts. Their weapons include both the ability to establish silent, life-lasting infections, the capture of host-derived genes to escape antiviral responses and the manipulation of host immunity at their own sake.

As an example, some herpesviruses, such as EBV and KSHV (HHV-8), express cytokine and chemokine homologous (e.g. vIL-6, vIL-10, MIP-1-like factors) that can switch host immunity from Th1 to Th2 [35-38]. The expression of a superantigen, encoded by some families of human ERVs, during development, can cause the deletion of reactive T cells and the restriction of the immune repertoire; when it is expressed during the acute infection of an exogenous retrovirus, the superantigen induces the aspecific stimulation of circulating B cells [39-41]. Intriguingly, the infections with some herpesviruses or with HIV were shown to affect the regulation of endogenous retroviruses, leading to increased inflammation, expression of superantigens and development of autoimmune diseases [42-46]. Therefore, the anti-cell

antibodies observed in HIV-exposed or -infected people can be triggered by different mechanisms, either exogenous or endogenous, which do not exclude the synergistic activities of other pathogens. On the other hand, anti-cell antibodies can develop from the exposure of cryptic epitopes or unusual conformations of self proteins, that can become “unknown” and thus “foreign” to mechanisms devoted to immune surveillance [47]. As examples of this latter mechanism, anti-CD4 antibodies are triggered by the unmasking of cryptic/conformational epitopes, that become exposed after gp120-CD4 binding, while anti-HLA responses depend on molecular mimicry between HIV glycoproteins and HLA domains; both these antibodies appear in response to HIV-antigens [48, 49].

Anti-CCR5 IgG and IgA have different ways of generation and different mechanisms of action. The N-terminus and the second external loop of CCR5 molecule host various immunodominant epitopes, which are crucial for chemokine and HIV binding; these domains are likely to be arranged in different conformations [50, 51]. Anti-CCR5 antibodies to the HIV binding site, i.e. the second external loop, appear in response to experimental immunization with cells expressing CCR5 or to HIV infection; these antibodies block HIV entry by binding competition or steric hindrance [52, 53]. Conversely, anti-CCR5 antibodies recognizing the first external loop of the protein can appear in response to HIV exposure, or even independently from it. These antibodies do not interfere with HIV binding directly, but induce co-receptor down-regulation, thus blocking virus infectivity by an indirect way [54-56].

4. CCR5 FUNCTIONS

CCR5 belongs to a large family of chemokine receptors, expressed on the surface of lymphocytes and other cell types, where are involved in signaling and coordination of the immune response [57]. Similarly to CXCR4, CCR5 is an HIV coreceptor [58-61]. CCR5 and other chemokine receptors belong to the family of seven transmembrane-domain proteins coupled to G proteins (G-protein coupled receptors, GPCRs), a very important family comprising many signaling receptors, such as rhodopsin and β -adrenergic receptors [62]. G-protein-coupled receptors are large transmembrane molecules and their three-dimensional structures are still poorly elucidated through physico-chemical spectroscopic methods, such as X-ray crystallography. Only the structures of rhodopsin, the two β -adrenergic receptors and adenosine receptor have been recently characterized [62]. The three-dimensional structure of the whole CCR5 molecule has not been solved yet. Existing information for small CCR5 peptides have been provided by NMR or crystallographic data, by homology modeling performed on similar structures and by indirect findings, e.g. structures for complexes between HIV envelope and monoclonal antibodies [62-64].

CCR5 is expressed on immature (Th0), memory and primed Th1 cells, on monocytes, macrophages and immature dendritic cells; on neurons, astrocytes and microglia; on epithelium, endothelium, vascular smooth muscle and fibroblasts [65]. Its preferential ligands are the pro-inflammatory cytokines CCL3 (MIP-1 α), CCL4 (MIP-1 β) and CCL5 (RANTES), involved in the initiation of effector response [66]. Other cytokines, such as CCL7 (MCP-3), CCL8 (MCP-

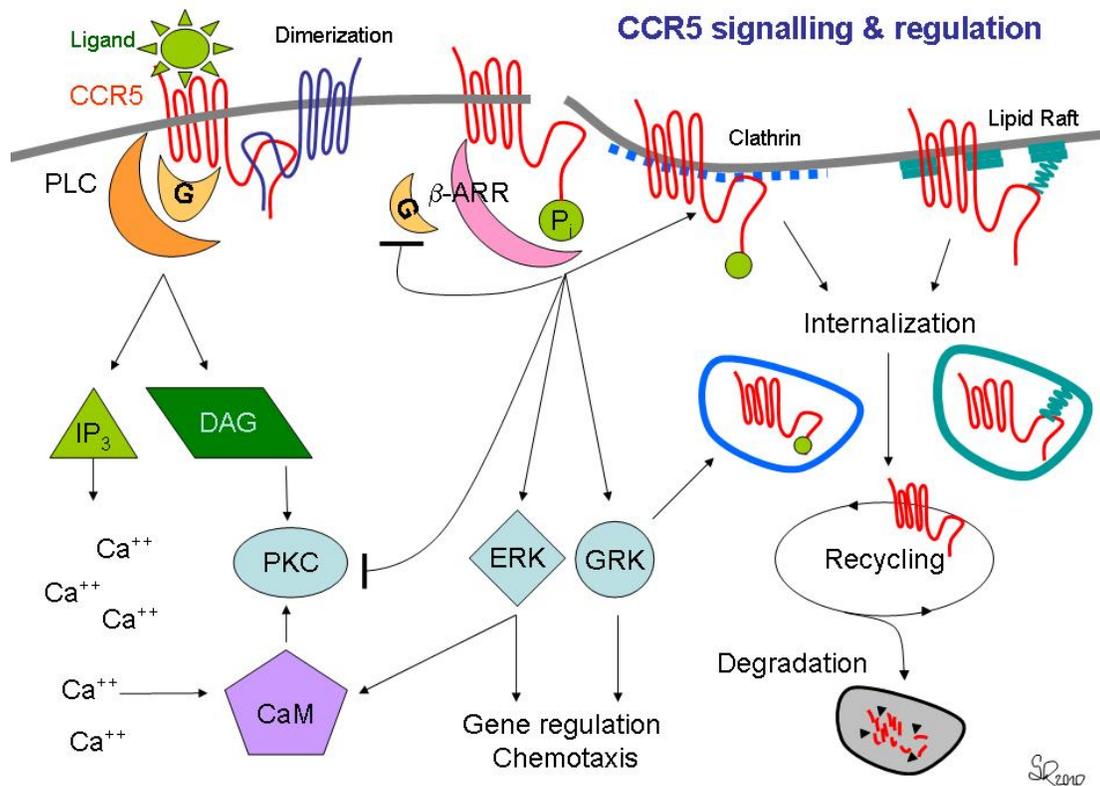


Fig. (1). CCR5 signaling and trafficking. Abbreviations: b-ARR, beta-arrestin; CaM, Calmodulin; DAG, diacylglycerol; ERK, Extracellular signal-regulated kinase; G, G protein; GRK, G protein-coupled receptor kinase; IP₃, inositol 1,4,5-trisphosphate; Pi, Phosphate; PKC, Protein Kinase C; PLC, phospholipase C.

2) and CCL13 (MCP-4), are, respectively, a competitive antagonist and two weak agonists. Chemokine binding may interfere with HIV docking, so natural CCR5 ligands were evaluated as HIV competitors, with different results: CCL3, CCL4, CCL5 and CCL8 displayed HIV-inhibiting properties, CCL7 was shown not to interfere, while CCL2 (MCP-1) even enhanced HIV infection *in vitro* [67]. CCL3L1 and CCL4L1 are variant chemokines encoded by genes with varying copy numbers. These chemokines inhibit CCR5 binding to HIV through allosteric blockade and receptor downregulation, in an inverse relationship; their gene copy numbers and hence their expression levels, can influence HIV progression [68].

Similarly to other GPCRs, CCR5 shows evidence of a complex regulation both at genetic and at macromolecular level. The organization of its unique gene may permit several ways of regulating gene transcription, RNA splicing and messenger translation. Exon 4 hosts the ORF, while exons 1-3 and the intercalating introns are transcribed and spliced to generate a family of messenger RNAs, all showing large 5'untranslated regions. Two different promoters, both lacking canonical TATA and CAAT motifs, can direct gene transcription; the former is a strong, regulated promoter located upstream the exon 1, while the latter is a weak, constitutive promoter placed between exons 1 and 3 [69]. Exon-1-containing transcripts, generated upon activation of the upstream inducible promoter, have been specifically isolated in activated T lymphocytes and in other cell types, as dendritic cells and monocytes undergoing chemokine stimulation and

antigen presentation [69, 70]. These cells coexpress both CCR5 and CXCR4 receptors, organized in homo- and heterodimers, as CCR5-CXCR4, CCR2-CCR5, CXCR4-Delta opioid receptor [71, 72]. According to FRET (*Fluorescence Resonance Energy Transfer*) assays, CCR5-CXCR4 heterodimers increased in response to MIP-1 α or RANTES binding, while SDF-1 reduced dimers density [72]. The state of dimerization can affect the response of any of the engaged receptors to their ligands; it is also required for receptor desensitization after chemokine signaling [73, 74]. CCR5 responsiveness to chemokines is further regulated through receptor desensitization, internalization and recycling, three phenomena occurring in different time frames — seconds to hours — after exposure to ligands. CCR5 signalling and coreceptor trafficking involve a cascade of protein kinases and second messengers, such as the protein kinase C (PKC) and the generation of a calcium flux, associated with RANTES-mediated chemotaxis. PKC-mediated phosphorylation of the receptor also promotes CCR5 binding to beta-arrestin and leads to its recycling and/or degradation [75]. Other details about CCR5 signaling and trafficking are summarized in Fig. (1).

Finally, CCR5 is the exclusive co-receptor mediating HIV entry through the mucosa, that is the scenario where most, if not all, horizontal and vertical HIV transmission take place [76, 77]. CXCR4 coreceptors are also expressed on the genital mucosa, but are very rare, since they are downregulated by the high SDF-1 local expression [78, 79].

CCR5-tropic (R5) viruses are preferentially transmitted upon interaction with the immune cells residing in submucosal tissues, as DCs, Langerhans cells and macrophages. Submucosal DCs subsequently transport HIV particles to regional lymph nodes, where they encounter — and can infect — CD4+ T cells [77].

5. CCR5 IS A SUITABLE IMMUNE TARGET

Due to its features and to its natural history, CCR5 is a key target in HIV therapy and prevention and has fostered therapeutic approaches to block HIV infection; to date, small-molecule inhibitors, chemically modified ligands and anti-CCR5 antibodies have shown antiviral properties in cell-based tests and in some *in vivo* trials [52, 80, 81]. Some drug inhibitors, such as Maraviroc, are already registered for the clinic use [81]. Other approaches, as the administration of Rapamycin or Statins, or the expression of anti-CCR5 intracellular antibodies or chemokines, have also been proposed to contain CCR5 receptors within target cells [82-85].

5.1. Natural antibodies To CCR5

As detailed above, different types of HIV-blocking antibodies to CCR5 have been isolated from HIV-infected and from HIV-exposed, seronegative (ESN) subjects. A peculiar type of these antibodies recognizes the first external loop of CCR5 receptor (ECL1), a domain not involved in ligand binding or in HIV docking. Anti-CCR5 antibodies have been only detected in serum and mucosal secretions from ESN people and in long-term non-progressing HIV-positive (LTNP) subjects, both men and women, supporting the hypothesis that these IgG and IgA are involved in HIV protection or in infection control [54, 86]. One clinical study searched for such anti-CCR5 antibodies in 497 subjects, including 85 LTNPs, 70 HIV-progressors, 135 HIV-positive patients receiving highly active antiretroviral therapy (HAART) and 207 HIV-negative donors [87]. Anti-CCR5 antibodies were isolated in 23% of the LTNP subjects but not in the other subpopulations studied ($P < .001$). Anti-CCR5 Abs recognized a conformational epitope within the ECL1 domain and induced a stable and long-lasting downregulation of CCR5 from the surface of T lymphocytes, thereby inhibiting HIV entry. Receptor internalization was specifically inhibited by sucrose, but not by filipin or nystatin, nocodazole or cytochalasin D, thus supporting a specific role for clathrin-coated pits and excluding the caveolae compartments [87]. In addition, CD4⁺ lymphocytes from the LTNP subpopulation who displayed anti-CCR5 Abs were resistant to *in vitro* infection with R5-tropic HIV-1 strains, due to CCR5 downregulation; anti-CCR5 antibodies were able to block *in vitro* infectivity of HIV primary isolates belonging to clades A, B and C. The level of anti-CCR5 antibodies appeared to be correlated with levels of HIV exposure, being lower in seronegative ESN subjects and higher in seropositive LTNP individuals (0.1% vs. 8% of the total antibodies, respectively).

Interestingly, the loss of anti-CCR5 antibodies was observed in the course of the clinical follow-up and this event was significantly associated with clinical progression toward disease in 9 out of 20 LTNP enrolled in the study; these LTNPs experienced a significant increase in viremia and required therapy, thus becoming “progressors”. Strikingly,

the other patients, who retained anti-CCR5 Abs, maintained a stable LTNP status without any treatment. According to the finding, the loss of anti-CCR5 Abs was associated with progression toward disease; this observation was strongly supported by the development of AIDS in some subjects despite antiretroviral therapy [87].

The persistence of very low, undetectable levels of HIV replication might provide a possible explanation for this unusual antibodies; the low, continuous antigen boost does not result in a strong generalized immune activation, similarly to what was observed in the course of natural latent viral infections (*e.g.*, herpesviruses) or in food-borne antigens and/or vaccines, which may establish tolerance and retain their antigenic potential [88, 89]. In the lucky subset of ESN and LTNP individuals who were able to control HIV, host physiological and immunological conditions might have established a positive feedback cycle that maintains undetectable levels of virus replication and a suitable antigen presentation on one hand and long-lasting responses, capable of blocking HIV through its major coreceptor on the other, therefore providing a key mechanism for fighting HIV replication [47]. Another key point in the study was the observation that the viral phenotype in LTNPs carrying anti-CCR5 antibodies did not change in the presence of such antibodies, thus confirming that the selective pressure of CCR5 inhibitors does not induce a change of viral phenotype *per se*, as already reported in a monkey model [90]. In addition, anti-CCR5 antibodies did not induce any apparent alterations in immune function, as demonstrated by the continued health status of subjects who retained anti-CCR5 antibodies; both these findings provide an argument against theoretical concerns about CCR5 targeting with specific antibodies.

5.2. mAbs to CCR5

Several studies have focused on the three-dimensional structure of the CCR5 receptor through the use of specific mAbs, defining epitopes involved in chemokine binding, receptor activation and trafficking and HIV coreceptor activity. Some monoclonal antibodies to CCR5, such as MC-1 or PA14, could also work as therapeutic inhibitors of viral entry, due to their ability in inhibiting gp120 binding and/or in promoting CCR5 internalization without triggering intracellular signaling; the humanized version of PA14, PRO140, has been tested in clinical studies [33, 34, 51]. A scheme representing CCR5 molecule, its binding domains and the key epitopes mapped on its structure is illustrated in Fig. (2).

Similarly to other GPCRs and membrane-associated proteins, CCR5 is poorly immunogenic; its four extracellular domains represent about one fourth of the whole sequence (90 out of 352 aminoacids); the two longer domains, the N-terminus and the second extracellular loop (ECL2) span about 30 aminoacids each [52]. These latter domains host immunodominant epitopes recognized by the majority of monoclonal antibodies [52]; both N-terminus and ECL2 domains are also involved in chemokine and HIV binding [51, 52, 91].

Alanine mapping and point mutation studies have mapped critical aminoacids on the CCR5 molecule, leading to design epitope maps and theoretical models representing the extracellular domains of the receptor and their hypothetical interactions [33, 51]. Not surprisingly, few mAbs were

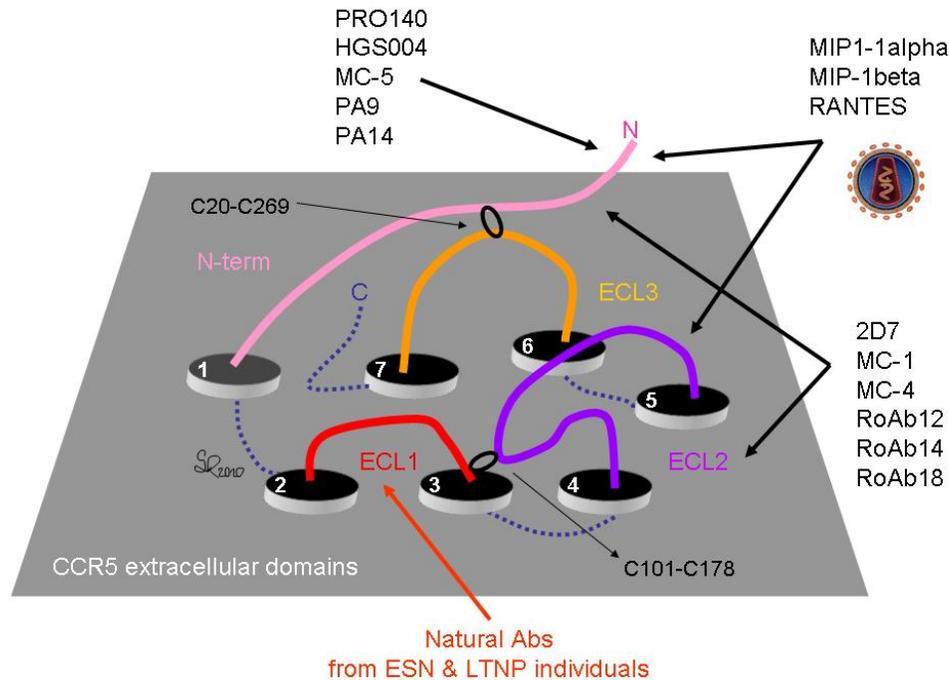


Fig. (2). Scheme illustrating the three-dimension structure of CCR5 coreceptor. Extracellular domains show HIV and chemokines binding sites and the domains mapped by mAbs and natural anti-CCR5 antibodies. C20-C269 and C101-C178 disulphide bonds are represented as black circles.

able to bind native and denatured CCR5 in Western Blot assays, a finding showing that most CCR5 epitopes are conformation-sensitive [91]. Key aminoacids included in discontinuous, conformational epitopes may embrace one or more residues among the first 20 aminoacids in the N-terminus, two distinct regions within the ECL2 domain and single aminoacids belonging to other domains, such as ECL1 [52]. 2D7, one of the most potent mAbs described in many studies, binds to the ECL2 domain [33, 51, 91]. Antibodies targeting the N-terminus domain of CCR5, as MC-5 or PA9, competed for binding of soluble gp120-CD4 complex with high affinity, but were less effective than the ECL2-specific mAbs in preventing cell-cell fusion and virus entry [33, 92]. Conversely, antibodies to ECL2 domain did not prevent gp120-CD4 complex binding effectively but were strong inhibitors of HIV entry; these findings supported a model of dual interaction between CCR5 and HIV, where the first interaction, involving the binding between V3 stem on the viral protein and the N-terminus of the coreceptor, occurred before the second one, which took V3 crown in close contact with the ECL2 domain and triggered HIV envelope-cell membrane fusion; both interactions with N-terminus and ECL2 domains were required for HIV docking [33, 52].

The ECL2 domain hosts both HIV- and chemokine-specific binding sites; antibodies recognizing this domain were also effective in preventing chemokine binding and/or signaling [91]. Antibodies recognizing conformational epitopes spanning different extracellular domains of the CCR5 molecule displayed different ability in inducing ligand binding, signaling and receptor trafficking (e.g. desensitization, phosphorylation, downregulation). For example, MC-6 antibody activated CCR5 but was unable to induce receptor in-

ternalization, whereas MC-1 caused CCR5 internalization, *via* cholesterol-rich raft domains; MC-4 specifically inhibited RANTES-mediated endocytosis, but did not affect chemokine signaling [51]. PA9 and PA12, all recognizing CCR5 N-terminus, were ineffective at blocking intracellular signaling, while PA14 and 2D7 prevented intracellular calcium mobilization induced by chemokine binding [33]. The wide spectrum of effects mediated by binding of the different mAbs supported the existence of multiple conformations for CCR5 molecules [51]. Most importantly, the modulation of specific events associated with the coreceptor, such as ligand binding, signaling and downregulation, opened the way to the use of mAbs as therapeutic tools, capable of preventing HIV spread by steric hindrance and/or receptor internalization without affecting physiologic chemokine signaling. Moreover, antiviral antibodies could also fight viruses by inducing antibody-dependent cellular cytotoxicity (ADCC), virus opsonization and by recruiting components of the complement cascade [93]. Another interesting feature shown by some mAbs was the possibility to obtain synergistic antiviral activity, due to the existence of various non-overlapping epitopes involved in HIV binding, docking and entry [94].

5.3. Passive immunization to CCR5

Humanized monoclonal antibodies recognizing CCR5 extracellular domains (the N-terminal and/or the second extracellular loop) have been developed and competed with gp120 binding [65]. Passive immunization with humanized mAbs may offer several advantages in respect to other antiviral drugs. MAbs are highly target-specific and therefore they minimize side effects or toxicity; their very long plasma

half-lives allow biweekly or even monthly administrations; mAbs are proteins administered intravenously, hence their pharmacokinetics, metabolism and toxicity differ from those of HIV-inhibiting drugs, that are low-molecular-weight molecules administered per os. Moreover, different anti-CCR5 mAbs can provide different spectra of antiviral and anti-chemotaxis activities. On the other hand, mAbs-based drugs also have disadvantages, such as the inconvenience of intravenous administration, the potential for inducing allergic reactions and the possible development of neutralizing anti-antibodies [94].

PRO140 and another mAb, HGS004, have been tested in HIV-infected subjects [95, 96]. At nanomolar concentrations *in vitro*, PRO140 blocked HIV strains belonging to different clades both in primary macrophages and in PBMC [34]. PRO140 inhibited HIV without blocking the CCR5 response to chemokines, whereas HGS004 prevented both viral infection and chemokine signaling. Notably, antibodies and small-molecule antagonists did not share the same mechanism and site of action; therefore, their activity might be synergic or contrasting and no cross-resistance were observed [65].

6. GENERATION OF ANTI-CCR5 IMMUNITY

Other experiments, carried out on mice and monkeys, showed that anti-CCR5 antibodies could be elicited in rodent, bird and monkey models and were also re-boosted when required [56, 97, 98]. Most importantly, anti-CCR5 IgG and IgA generated by immunization shared HIV-blocking properties with human monoclonal immunoglobulins and with natural antibodies found in exposed individuals [54, 56].

Immunization experiments and *in vitro* studies of elicited antibodies were performed by Chain *et al.* [99], who immunized rabbits with chimeric peptides corresponding to a very short fragment of the N-terminal sequence of CCR5 and with a T-specific peptide from *Tetanus* toxoid. T-specific CCR5 epitopes were not included in the immunogen to prevent the development of host autoimmune responses. Immunization generated a strong antibody response; binding experiments to N-terminal and full-length CCR5 suggested that CCR5-binding antibodies were a small percentage of the total antibodies elicited by immunization; nevertheless, anti-CCR5 specific antibodies blocked HIV infection of macrophages *in vitro*. Devito *et al.* [100] carried out a long-term immunization with an intranasal DNA prime followed by a peptide booster immunization. Delivered antigens were peptides from gp120 V3 loop, gp41 (MPER peptides containing the ELDKWAS epitope) and CCR5-ECL2 domain. The vaccination schedule elicited specific IgG and IgA in sera and in mucosal secretions (intestinal, vaginal and lung) in immunized mice. More interestingly, long-term IgG and IgA responses were still observed after 12 months from boosting both in serum and in mucosal secretions. HIV-blocking Abs were still detected in serum 12 months after boosting. According to this study, intranasal DNA prime followed by one peptide/L3 adjuvant booster immunization, but not vice versa, induced long-lasting HIV-blocking Abs and B memory cells to poorly immunogenic, conformational epitopes. Barassi *et al.* [56] generated chimeric immunogens containing a CCR5 peptide from the first extracellular domain

(ECL1) in the context of the capsid protein of flock house virus, a conformation-constrained expression system [101]. Administered to mice by systemic or mucosal route, the immunogens elicited anti-CCR5 IgG and IgA both in sera and in vaginal fluids. Similarly to HIV-exposed seronegative individuals, mice producing anti-CCR5 autoantibodies expressed significantly reduced levels of CCR5 on the surfaces of CD4⁺ cells from peripheral blood and vaginal washes. *In vitro* studies showed that murine IgG and IgA (i) specifically bound human and mouse CD4⁺ lymphocytes and the CCR5-transfected U87 cell line; (ii) downregulated CCR5 expression of CD4⁺ cells from both humans and untreated mice; (iii) inhibited CCL4/MIP-1 β chemotaxis of CD4⁺ CCR5⁺ lymphocytes and (iv) blocked *in vitro* infectivity of HIV R5 strains belonging to clade B. Finally, Pastori *et al.* [102] performed a peptide-scanning assay on a panel of synthetic peptides spanning the CCR5-ECL1 region; the resulting peptides were assayed with a pool of natural anti-CCR5 antibodies and used to immunize mice and chickens. Further structural characterization of the peptides was provided by NMR spectroscopy and by molecular dynamics simulations. Amino acid substitutions in positions 95 and 96 increased antibody-peptide binding compared to the wild-type peptide. The A95-A96 peptide was shown to induce, in mice and chickens, antibodies displaying biological activity at very low concentrations. Strikingly, chicken antibodies to the modified peptide specifically recognized human CCR5 molecules, downregulated receptors from lymphocytes, inhibited CCR5-dependent chemotaxis and prevented infection by several R5 primary isolates belonging to Clades A, B, C and E, displaying IC₅₀ values lower than 3 ng/ml. NMR spectroscopy and molecular dynamics simulations confirmed the high flexibility of isolated epitopes and suggested that A95-A96 substitutions conferred a slightly higher tendency to generate helical conformations combined with a lower steric hindrance of the side chains in the peptides. The different structural behavior of the mutagenized loop might account for a better molecular structural organization, allowing the induction of the fittest antibodies. Optimized antibodies recognized and bound native CCR5 with higher affinity and displayed enhanced biological activity.

Other *in vivo* studies coupled immunization experiments with *in vivo* challenges of vaccinated animals to evaluate whether a break in B-tolerance was achieved and what was the extent of immune protection conferred by tested immunogens. Chackerian *et al.* [97] used the N-terminal domain of pigtailed macaque CCR5 fused to Streptavidin. Once conjugated at high densities to capsid protein L1 within bovine papilloma virus-like particles, this immunogen induced high-titer anti-CCR5 IgG that blocked infection by CCR5-tropic simian-human immunodeficiency virus (SHIV) *in vitro*. FACS analysis of spleen cells, thymus cells and PBMC did not detect any decline in the number of CCR5-expressing cells (T lymphocytes and macrophages) in immunized animals vs controls. In SHIV-challenged macaques, viral loads and time to control of viremia were significantly decreased in respect to controls, indicating that CCR5 autoantibodies could have contributed to the control of viral replication. Bogers *et al.* [103] assayed a vaccine consisting of three extracellular peptides of CCR5, an N-terminal HIV gp120 fragment generated in transgenic plants and the recombinant simian immunodeficiency virus p27. They were linked to the

microbial heat-shock protein HSP70, used as a carrier and the vaccine was administered by mucosal and systemic routes. Vaginal challenge with SHIV infected all macaques, with a significant variation in viral loads between the immunized and control animals; the virus was cleared in five of nine immunized animals. Misumi *et al.* [104] adopted synthetic cyclic peptides from the second external loop to induce anti-CCR5 antibodies in cynomolgus macaques. The immunization with a conjugated multiple-Ag peptide (cyclic closed chain dodecapeptide, cDDR5-MAP) induced long-lasting anti-cDDR5 antibodies reacting with both human and macaque CCR5 molecules, which suppressed infections by an R5 HIV-1 laboratory isolate (HIV JRFL), by R5 HIV-1 primary isolates (clade A:HIV 93RW004 and clade C:HIV MJ4) and by a pathogenic simian/HIV (SHIV SF162P3) bulk isolate *in vitro*. After SHIV challenge, vaccinated cynomolgus macaques showed an attenuated acute infection and a lower viral load than unvaccinated control animals.

According to *in vitro* and *in vivo* findings, immunization did elicit antibodies endowed with blocking properties, effectively destroying B-tolerance. Despite the fact that none of the immunogens assayed *in vivo* was able to confer full protection from virus challenge, the infection of vaccinated subjects was lower than in the controls and virus control was achieved in most subjects. Finally, *in vitro* studies also showed that conformational changes in the CCR5 protein, together with host factors, had the potential to modulate protein immunogenicity *in vivo* and might also play a role in the natural resistance to HIV infection.

9. CONCLUSION AND PERSPECTIVES

CCR5 is a key player in HIV entry and many attempts to prevent its role in infection have been developed and assayed. The clinical use of small CCR5 inhibitors has proven the feasibility and the efficacy of CCR5 targeting, but it has also raised concerns about the safety of this approach: drug-resistant R5 HIV strains have been isolated in cell cultures and in patients receiving Maraviroc and other CCR5 inhibitors [80, 81, 105]. The use of humanized monoclonal antibodies has proven effective, safe and tough in HIV-infected patients, suggesting that passive immunization may offer therapeutic advantages [95, 96]. The use of engineered chemokines induced receptor downregulation, removing CCR5 from availability for HIV binding; despite its effectiveness, this approach might be associated *in vivo* with adverse inflammatory events [106]. An HIV vaccine remains the most expected goal to be accomplished in HIV research, showing its value both in therapeutic intervention and in prevention [107]. Vaccination may offer long-lasting protection with few administrations, in a way acceptable in many geographical and social contexts, where other forms of prevention for sexually transmitted diseases could be impractical or rejected [108].

Anti-CCR5 vaccination is an innovative anti-HIV strategy, which could provide effective protection or safe containment to virus spread. Most importantly, anti-CCR5 antibodies raised in animal models or naturally occurring upon HIV exposure showed blocking activity to different virus clades, a result that is hardly achieved by conventional HIV-based immunogens [56, 87, 97]. Indeed, the feasibility of anti-CCR5 vaccination has been already demonstrated by

two groups of naturally CCR5-deficient people. Individuals deprived of CCR5 receptor by genetic deletion [109-111] and those carrying naturally occurring anti-CCR5 antibodies downregulating the receptor *in vivo* [54, 86, 87] were found to be healthy and largely resistant to HIV-infection. Importantly, natural anti-CCR5 antibodies to the ECL1 domain have been uniquely observed in the sera and in mucosal fluids of individuals who remained uninfected despite repeated and unprotected sexual exposure to HIV and in HIV-infected individuals with long-term, asymptomatic infection. The finding that both ESN and LTNP subpopulations exerted a high and durable control on the virus confirmed the hypothesis that natural anti-CCR5 antibodies could be associated with protection. This concept was further strengthened by the good health and immune status shown by the LTNP cohort, confirming that long-lasting CCR5 downregulation was not harmful; conversely, cohort follow-up showed that the loss of anti-CCR5 responses experienced by some patients was associated with a decline in virus control [87]. These findings are noteworthy because genetic CCR5 deletion has been associated with an increased susceptibility to some viral and bacterial pathogens [112]; moreover, anti-self immunity was one of the mechanisms evoked to explain the generation of natural anti-CCR5 antibodies [47] and a possible adverse event associated with anti-CCR5 vaccination [65]. Conversely, CCR5 targeting could offer therapeutic advantages in some autoimmune diseases, as rheumatoid arthritis [113], or in transplantation therapy, all situations where chemokine signaling and cell recruitment are immune mechanisms sustaining tissue damage [114]. Another key finding from the follow-up of the LTNP cohort was the lack of an R5-to-X4 tropism shift, a fact supporting the safety of antibody-mediated coreceptor targeting [87]; this is a key point to be considered, due to the concerns raised by the therapeutic use of small-molecule CCR5 inhibitors, which are prone to *in vitro* and *in vivo* drug resistance and might favor the selection of dual-tropic or X4-tropic virus strains [81, 90, 115]. Indeed, immunization experiments performed in animal models have shown that anti-CCR5 antibodies can be obtained *in vivo*, provided that suitable vector systems are used, either to break B-tolerance to the self-CCR5 antigen and to constrain the ECL1 peptide (i.e. the target domain of these natural anti-CCR5 antibodies) in a conformation similar to the naturally occurring, immunogenic one [56, 102]. Moreover, anti-CCR5 antibodies elicited by the mucosal route are long-lasting and can be promptly re-boosted upon immunization, either in sera or, most importantly, in mucosal fluids, showing the feasibility of local immunity at major portals of HIV entry [56].

Taken together, all of the findings reviewed here support the significance of interventions focused on CCR5 in its role as principal HIV coreceptor. Among all strategies now available or under development, naturally occurring anti-CCR5 antibodies show the therapeutic potential to provide durable, effective and safe systemic and, especially, local immunity to HIV. As shown by follow-up studies and immunization experiments, antibody-mediated CCR5 targeting was not only feasible but it was also well tolerated. Together with other immune-modulating strategies, this unconventional approach could open unprecedented avenues of treatment not only for HIV/AIDS but also for other disorders where harmful pro-inflammatory responses can develop.

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REFERENCES

- [1] Daniel V, Schimpf K, Opelz G. Lymphocyte autoantibodies and alloantibodies in HIV-positive haemophilia patients. *Clin Exp Immunol* 1989; 75: 178-83.
- [2] Dorsett B, Cronin W, Chuma V, Ioachim HL. Anti-lymphocyte antibodies in patients with the acquired immune deficiency syndrome. *Am J Med* 1985; 78: 621-6.
- [3] Lutz HU, Flepp R, Stringaro-Wipf G. Naturally occurring autoantibodies to exoplasmic and cryptic regions of band 3 protein, the major integral membrane protein of human red blood cells. *J Immunol* 1984; 133: 2610-8.
- [4] Blanton M, Balakrishnan K, Dumaswala U, Zelenski K, Greenwalt TJ. HLA antibodies in blood donors with reactive screening tests for antibody to the immunodeficiency virus. *Transfusion* 1987; 27: 118-9.
- [5] Tiberio L, Caruso A, Pozzi A, *et al.* The detection and biological activity of human antibodies to IL-2 in normal donors. *Scand J Immunol* 1993; 38: 472-6.
- [6] Horn MP, Gerster T, Ochensberger B, *et al.* Human anti-FcepsilonRIalpha autoantibodies isolated from healthy donors cross-react with tetanus toxoid. *Eur J Immunol* 1999; 29: 1139-48.
- [7] Peters B, Whittall T, Babaahmady K, *et al.* Effect of heterosexual intercourse on mucosal alloimmunisation and resistance to HIV-1 infection. *Lancet* 2004; 363: 518-24.
- [8] Spruth M, Stoiber H, Kacani L, Schonitzer D, Dierich MP. Neutralization of HIV type 1 by alloimmune sera derived from poly-transfused patients. *AIDS Res Hum Retroviruses* 1999; 15: 533-43.
- [9] Lehner T, Bergmeier L, Wang Y, Tao L, Mitchell E. A rational basis for mucosal vaccination against HIV infection. *Immunol Rev* 1999; 170: 183-96.
- [10] Arthur LO, Bess JW, Jr, Sowder RC, 2nd, *et al.* Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. *Science* 1992; 258: 1935-8.
- [11] Frank I, Stoiber H, Godar S, *et al.* Acquisition of host cell-surface-derived molecules by HIV-1. *Aids* 1996; 10: 1611-20.
- [12] Lehner T, Shearer GM, Hackett CJ, Schultz A, Sharma OK. Alloimmunization as a strategy for vaccine design against HIV/AIDS. *AIDS Res Hum Retroviruses* 2000; 16: 309-13.
- [13] Leith JG, Clark DA, Matthews TJ, *et al.* Assessing human alloimmunization as a strategy for inducing HIV type 1 neutralizing anti-HLA responses. *AIDS Res Hum Retroviruses* 2003; 19: 957-65.
- [14] Kingsley C, Peters B, Babaahmady K, *et al.* Heterosexual and homosexual partners practising unprotected sex may develop allogeneic immunity and to a lesser extent tolerance. *PLoS One* 2009; 4: e7938.
- [15] Mayer-Siuta R, Keil LB, DeBari VA. Autoantibodies and circulating immune complexes in subjects infected with human immunodeficiency virus. *Med Microbiol Immunol (Berl)* 1988; 177: 189-94.
- [16] Conte R, Giovanardi L, Tazzari PL, *et al.* Non-HLA lymphocytotoxic antibodies in HIV-seropositive and HIV-seronegative hemophiliacs. *Vox Sang* 1988; 54: 47-51.
- [17] Karparkin S, Nardi M. Autoimmune anti-HIV-1gp120 antibody with antiidiotypic-like activity in sera and immune complexes of HIV-1-related immunologic thrombocytopenia. *J Clin Invest* 1992; 89: 356-64.
- [18] Borghi MO, De Santis C, Barcellini W, *et al.* Autoantibodies against beta 2-microglobulin-free HLA antigens in AIDS patients. *J Acquir Immune Defic Syndr* 1993; 6: 1114-9.
- [19] Ditzel HJ, Barbas SM, Barbas CF, 3rd, Burton DR. The nature of the autoimmune antibody repertoire in human immunodeficiency virus type 1 infection. *Proc Natl Acad Sci USA* 1994; 91: 3710-4.
- [20] De Santis C, Robbioni P, Longhi R, *et al.* Cross-reactive response to human immunodeficiency virus type 1 (HIV-1) gp120 and HLA class I heavy chains induced by receipt of HIV-1-derived envelope vaccines. *J Infect Dis* 1993; 168: 1396-403.
- [21] Burastero SE, Gaffi D, Lopalco L, *et al.* Autoantibodies to CD4 in HIV type 1-exposed seronegative individuals. *AIDS Res Hum Retroviruses* 1996; 12: 273-80.
- [22] Lopalco L, Pastori C, Cosma A, *et al.* Anti-cell antibodies in exposed seronegative individuals with HIV type 1-neutralizing activity. *AIDS Res Hum Retroviruses* 2000; 16: 109-15.
- [23] Dominguez V, Gevorkian G, Govezensky T, *et al.* Antigenic homology of HIV-1 GP41 and human platelet glycoprotein GPIIb3 (integrin beta3). *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; 17: 385-90.
- [24] Golding H, Shearer GM, Hillman K, *et al.* Common epitope in human immunodeficiency virus (HIV) I-GP41 and HLA class II elicits immunosuppressive autoantibodies capable of contributing to immune dysfunction in HIV I-infected individuals. *J Clin Invest* 1989; 83: 1430-5.
- [25] Pinter C, Siccardi AG, Lopalco L, Longhi R, Clivio A. HIV glycoprotein 41 and complement factor H interact with each other and share functional as well as antigenic homology. *AIDS Res Hum Retroviruses* 1995; 11: 971-80.
- [26] Serres PF. Molecular mimicry between the trimeric ectodomain of the transmembrane protein of immunosuppressive lentiviruses (HIV-SIV-FIV) and interleukin 2. *C R Acad Sci III* 2000; 323: 1019-29.
- [27] Serres PF. AIDS: an immune response against the immune system. Role of a precise tridimensional molecular mimicry. *J Autoimmun* 2001; 16: 287-91.
- [28] Spehar T, Strand M. Molecular mimicry between HIV-1 gp41 and an astrocyte isoform of alpha-actinin. *J Neurovirol* 1995; 1: 381-90.
- [29] Stoiber H, Thielens NM, Ebenbichler C, Arlaud GJ, Dierich MP. The envelope glycoprotein of HIV-1 gp120 and human complement protein C1q bind to the same peptides derived from three different regions of gp41, the transmembrane glycoprotein of HIV-1, and share antigenic homology. *Eur J Immunol* 1994; 24: 294-300.
- [30] Stott EJ. Anti-cell antibody in macaques. *Nature* 1991; 353: 393.
- [31] Bergmeier L, Babaahmady K, Wang Y, Lehner T. Mucosal alloimmunization elicits T-cell proliferation, CC chemokines, CCR5 antibodies and inhibition of simian immunodeficiency virus infectivity. *J Gen Virol* 2005; 86: 2231-8.
- [32] Dela Cruz CS, MacDonald KS, Barber BH. Anti-major histocompatibility complex antibody responses in macaques *via* intradermal DNA immunizations. *Vaccine* 2000; 18: 3152-65.
- [33] Olson WC, Rabut GE, Nagashima KA, *et al.* Differential inhibition of human immunodeficiency virus type 1 fusion, gp120 binding, and CC-chemokine activity by monoclonal antibodies to CCR5. *J Virol* 1999; 73: 4145-55.
- [34] Trkola A, Ketas TJ, Nagashima KA, *et al.* Potent, broad-spectrum inhibition of human immunodeficiency virus type 1 by the CCR5 monoclonal antibody PRO 140. *J Virol* 2001; 75: 579-88.
- [35] Stine JT, Wood C, Hill M, *et al.* KSHV-encoded CC chemokine vMIP-III is a CCR4 agonist, stimulates angiogenesis, and selectively chemoattracts TH2 cells. *Blood* 2000; 95: 1151-7.
- [36] Endres MJ, Garlisi CG, Xiao H, Shan L, Hedrick JA. The Kaposi's sarcoma-related herpesvirus (KSHV)-encoded chemokine vMIP-I is a specific agonist for the CC chemokine receptor (CCR)8. *J Exp Med* 1999; 189: 1993-8.
- [37] Colantonio L, Recalde H, Sinigaglia F, D'Ambrosio D. Modulation of chemokine receptor expression and chemotactic responsiveness during differentiation of human naive T cells into Th1 or Th2 cells. *Eur J Immunol* 2002; 32: 1264-73.
- [38] Singh UP, Singh S, Ravichandran P, Taub DD, Lillard JW, Jr. Viral macrophage-inflammatory protein-II: a viral chemokine that differentially affects adaptive mucosal immunity compared with its mammalian counterparts. *J Immunol* 2004; 173: 5509-16.
- [39] Hoffmann MK, Dannecker G, Mecheri S. Regulation of T cell function by Mtv-7 gene products. *Semin Immunol* 1992; 4: 337-51.
- [40] Meylan F, De Smedt M, Leclercq G, *et al.* Negative thymocyte selection to HERV-K18 superantigens in humans. *Blood* 2005; 105: 4377-82.
- [41] Nakagawa K, Harrison LC. The potential roles of endogenous retroviruses in autoimmunity. *Immunol Rev* 1996; 152: 193-236.
- [42] Contreras-Galindo R, Lopez P, Velez R, Yamamura Y. HIV-1 infection increases the expression of human endogenous retroviruses type K (HERV-K) *in vitro*. *AIDS Res Hum Retroviruses* 2007; 23: 116-22.

- [43] Tai AK, Luka J, Ablashi D, Huber BT. HHV-6A infection induces expression of HERV-K18-encoded superantigen. *J Clin Virol* 2009; 46: 47-8.
- [44] Brudek T, Christensen T, Hansen HJ, Petersen T, Moller-Larsen A. Synergistic immune responses induced by endogenous retrovirus and herpesvirus antigens result in increased production of inflammatory cytokines in multiple sclerosis patients. *Scand J Immunol* 2008; 67: 295-303.
- [45] Brudek T, Luhdorf P, Christensen T, Hansen HJ, Moller-Larsen A. Activation of endogenous retrovirus reverse transcriptase in multiple sclerosis patient lymphocytes by inactivated HSV-1, HHV-6 and VZV. *J Neuroimmunol* 2007; 187: 147-55.
- [46] Ruprecht K, Obojes K, Wengel V, *et al.* Regulation of human endogenous retrovirus W protein expression by herpes simplex virus type 1: implications for multiple sclerosis. *J Neurovirol* 2006; 12: 65-71.
- [47] Russo S, Lopalco L. Is autoimmunity a component of natural immunity to HIV? *Curr HIV Res* 2006; 4: 177-90.
- [48] Beretta A, Furci L, Burastero S, *et al.* HIV-1-specific immunity in persistently seronegative individuals at high risk for HIV infection. *Immunol Lett* 1996; 51: 39-43.
- [49] Furci L, Beretta A, Siccardi A, *et al.* Human immunodeficiency virus type 1 glycoprotein 120-specific T lymphocytes provide intermolecular help for anti-CD4 autoantibody production in exposed uninfected subjects. *AIDS Res Hum Retroviruses* 1997; 13: 1461-9.
- [50] Bacon KB, Premack BA, Gardner P, Schall TJ. Activation of dual T cell signaling pathways by the chemokine RANTES. *Science* 1995; 269: 1727-30.
- [51] Blanpain C, Vanderwinden JM, Cihak J, *et al.* Multiple active states and oligomerization of CCR5 revealed by functional properties of monoclonal antibodies. *Mol Biol Cell* 2002; 13: 723-37.
- [52] Olson WC, Jacobson JM. CCR5 monoclonal antibodies for HIV-1 therapy. *Curr Opin HIV AIDS* 2009; 4: 104-11.
- [53] Bouhhal H, Latry V, Requena M, *et al.* Natural antibodies to CCR5 from breast milk block infection of macrophages and dendritic cells with primary R5-tropic HIV-1. *J Immunol* 2005; 174: 7202-9.
- [54] Lopalco L, Barassi C, Pastori C, *et al.* CCR5-reactive antibodies in seronegative partners of HIV-seropositive individuals down-modulate surface CCR5 *in vivo* and neutralize the infectivity of R5 strains of HIV-1 *In vitro*. *J Immunol* 2000; 164: 3426-33.
- [55] Lopalco L. Humoral immunity in HIV-1 Exposure: cause or effect of HIV resistance? *Curr HIV Res* 2004; 2: 79-92.
- [56] Barassi C, Soprana E, Pastori C, *et al.* Induction of murine mucosal CCR5-reactive antibodies as an anti-human immunodeficiency virus strategy. *J Virol* 2005; 79: 6848-58.
- [57] Huttenrauch F, Pollok-Kopp B, Oppermann M. G protein-coupled receptor kinases promote phosphorylation and beta-arrestin-mediated internalization of CCR5 homo- and hetero-oligomers. *J Biol Chem* 2005; 280: 37503-15.
- [58] Alkhatib G, Combadiere C, Broder CC, *et al.* CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 1996; 272: 1955-8.
- [59] Deng H, Liu R, Ellmeier W, *et al.* Identification of a major coreceptor for primary isolates of HIV-1. *Nature* 1996; 381: 661-6.
- [60] Dragic T, Litwin V, Allaway GP, *et al.* HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 1996; 381: 667-73.
- [61] Balter, M. A second coreceptor for HIV in early stages of infection. *Science* 1996; 272: 1740.
- [62] Lodowski DT, Palczewski K. Chemokine receptors and other G protein-coupled receptors. *Curr Opin HIV AIDS* 2009; 4: 88-95.
- [63] Huang CC, Lam SN, Acharya P, *et al.* Structures of the CCR5 N terminus and of a tyrosine-sulfated antibody with HIV-1 gp120 and CD4. *Science* 2007; 317: 1930-4.
- [64] Lam SN, Acharya P, Wyatt R, Kwong PD, Bewley CA. Tyrosine-sulfate isosteres of CCR5 N-terminus as tools for studying HIV-1 entry. *Bioorg Med Chem* 2008; 16: 10113-20.
- [65] Corbeau P, Reynes J. CCR5 antagonism in HIV infection: ways, effects, and side effects. *Aids* 2009; 23: 1931-43.
- [66] Trifilo MJ, Bergmann CC, Kuziel WA, Lane TE. CC chemokine ligand 3 (CCL3) regulates CD8(+)-T-cell effector function and migration following viral infection. *J Virol* 2003; 77: 4004-14.
- [67] Alkhatib G. The biology of CCR5 and CXCR4. *Curr Opin HIV AIDS* 2009; 4: 96-103.
- [68] Gonzalez E, Kulkarni H, Bolivar H, *et al.* The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* 2005; 307: 1434-40.
- [69] Mummidi S, Ahuja SS, McDaniel BL, Ahuja SK. The human CC chemokine receptor 5 (CCR5) gene. Multiple transcripts with 5'-end heterogeneity, dual promoter usage, and evidence for polymorphisms within the regulatory regions and noncoding exons. *J Biol Chem* 1997; 272: 30662-71.
- [70] Mummidi S, Adams LM, VanCompernelle SE, *et al.* Production of specific mRNA transcripts, usage of an alternate promoter, and octamer-binding transcription factors influence the surface expression levels of the HIV coreceptor CCR5 on primary T cells. *J Immunol* 2007; 178: 5668-81.
- [71] Mellado M, Rodriguez-Frade JM, Vila-Coro AJ, *et al.* Chemokine receptor homo- or heterodimerization activates distinct signaling pathways. *Embo J* 2001; 20: 2497-507.
- [72] Isik N, Hereld D, Jin T. Fluorescence resonance energy transfer imaging reveals that chemokine-binding modulates heterodimers of CXCR4 and CCR5 receptors. *PLoS One* 2008; 3: e3424.
- [73] Hereld D, Jin T. Slamming the DOR on chemokine receptor signaling: heterodimerization silences ligand-occupied CXCR4 and delta-opioid receptors. *Eur J Immunol* 2008; 38: 334-7.
- [74] Venkatesan S, Petrovic A, Locati M, *et al.* A membrane-proximal basic domain and cysteine cluster in the C-terminal tail of CCR5 constitute a bipartite motif critical for cell surface expression. *J Biol Chem* 2001; 276: 40133-45.
- [75] Oppermann M. Chemokine receptor CCR5: insights into structure, function, and regulation. *Cell Signal* 2004; 16: 1201-10.
- [76] Bomsel M, Alfsen A. Entry of viruses through the epithelial barrier: pathogenic trickery. *Nat Rev Mol Cell Biol* 2003; 4: 57-68.
- [77] Margolis L, Shattock R. Selective transmission of CCR5-utilizing HIV-1: the 'gatekeeper' problem resolved? *Nat Rev Microbiol* 2006; 4: 312-7.
- [78] Agace WW, Amara A, Roberts AI, *et al.* Constitutive expression of stromal derived factor-1 by mucosal epithelia and its role in HIV transmission and propagation. *Curr Biol* 2000; 10: 325-8.
- [79] Verani A, Lusso P. Chemokines as natural HIV antagonists. *Curr Mol Med* 2002; 2: 691-702.
- [80] Gulick RM, Lalezari J, Goodrich J, *et al.* Maraviroc for previously treated patients with R5 HIV-1 infection. *N Engl J Med* 2008; 359: 1429-41.
- [81] Moore JP, Kuritzkes DR. A piece de resistance: how HIV-1 escapes small molecule CCR5 inhibitors. *Curr Opin HIV AIDS* 2009; 4: 118-24.
- [82] Heredia A, Gilliam B, Latinovic O, *et al.* Rapamycin reduces CCR5 density levels on CD4 T cells, and this effect results in potentiation of enfuvirtide (T-20) against R5 strains of human immunodeficiency virus type 1 *in vitro*. *Antimicrob Agents Chemother* 2007; 51: 2489-96.
- [83] Nabatov AA, Pollakis G, Linnemann T, Paxton WA, de Baar MP. Statins disrupt CCR5 and RANTES expression levels in CD4+ T lymphocytes *in vitro* and preferentially decrease infection of R5 versus X4 HIV-1. *PLoS One* 2007; 2: e470.
- [84] Steinberger P, Andris-Widhopf J, Buhler B, Torbett BE, Barbas CF, 3rd. Functional deletion of the CCR5 receptor by intracellular immunization produces cells that are refractory to CCR5-dependent HIV-1 infection and cell fusion. *Proc Natl Acad Sci USA* 2000; 97: 805-10.
- [85] Yang AG, Bai X, Huang XF, Yao C, Chen S. Phenotypic knockout of HIV type 1 chemokine coreceptor CCR-5 by intrakines as potential therapeutic approach for HIV-1 infection. *Proc Natl Acad Sci USA* 1997; 94: 11567-72.
- [86] Barassi C, Lazzarin A, Lopalco L. CCR5-specific mucosal IgA in saliva and genital fluids of HIV-exposed seronegative subjects. *Blood* 2004; 104: 2205-6.
- [87] Pastori C, Weiser B, Barassi C, *et al.* Long-lasting CCR5 internalization by antibodies in a subset of long-term nonprogressors: a possible protective effect against disease progression. *Blood* 2006; 107: 4825-33.
- [88] Bourinbaier AS, Metadilgkul O, Jirathitikal V. Mucosal AIDS vaccines. *Viral Immunol* 2003; 16: 427-45.
- [89] Oxman MN. Herpes zoster pathogenesis and cell-mediated immunity and immunosenescence. *J Am Osteopath Assoc* 2009; 109: S13-7.

- [90] Wolinsky SM, Veazey RS, Kunstman KJ, *et al.* Effect of a CCR5 inhibitor on viral loads in macaques dual-infected with R5 and X4 primate immunodeficiency viruses. *Virology* 2004; 328: 19-29.
- [91] Lee B, Sharon M, Blanpain C, *et al.* Epitope mapping of CCR5 reveals multiple conformational states and distinct but overlapping structures involved in chemokine and coreceptor function. *J Biol Chem* 1999; 274: 9617-26.
- [92] Zhang J, Rao E, Dioszegi M, *et al.* The second extracellular loop of CCR5 contains the dominant epitopes for highly potent anti-human immunodeficiency virus monoclonal antibodies. *Antimicrob Agents Chemother* 2007; 51: 1386-97.
- [93] Huber M, Olson WC, Trkola A. Antibodies for HIV treatment and prevention: window of opportunity? *Curr Top Microbiol Immunol* 2008; 317: 39-66.
- [94] Ji C, Brandt M, Dioszegi M, *et al.* Novel CCR5 monoclonal antibodies with potent and broad-spectrum anti-HIV activities. *Antiviral Res* 2007; 74: 125-37.
- [95] Jacobson JM, Saag MS, Thompson MA, *et al.* Antiviral activity of single-dose PRO 140, a CCR5 monoclonal antibody, in HIV-infected adults. *J Infect Dis* 2008; 198: 1345-52.
- [96] Lalezari J, Yadavalli GK, Para M, *et al.* Safety, pharmacokinetics, and antiviral activity of HGS004, a novel fully human IgG4 monoclonal antibody against CCR5, in HIV-1-infected patients. *J Infect Dis* 2008; 197: 721-7.
- [97] Chackerian B, Lowy DR, Schiller JT. Induction of autoantibodies to mouse CCR5 with recombinant papillomavirus particles. *Proc Natl Acad Sci USA* 1999; 96: 2373-8.
- [98] Chackerian B, Briglio L, Albert PS, Lowy DR, Schiller JT. Induction of autoantibodies to CCR5 in macaques and subsequent effects upon challenge with an R5-tropic simian/human immunodeficiency virus. *J Virol* 2004; 78: 4037-47.
- [99] Chain BM, Noursadeghi M, Gardener M, Tsang J, Wright E. HIV blocking antibodies following immunisation with chimaeric peptides coding a short N-terminal sequence of the CCR5 receptor. *Vaccine* 2008; 26: 5752-9.
- [100] Devito C, Zuber B, Schroder U, *et al.* Intranasal HIV-1-gp160-DNA/gp41 peptide prime-boost immunization regimen in mice results in long-term HIV-1 neutralizing humoral mucosal and systemic immunity. *J Immunol* 2004; 173: 7078-89.
- [101] Buratti E, McLain L, Tisminetzky S, *et al.* The neutralizing antibody response against a conserved region of human immunodeficiency virus type 1 gp41 (amino acid residues 731-752) is uniquely directed against a conformational epitope. *J Gen Virol* 1998; 79(Pt 11): 2709-16.
- [102] Pastori C, Clivio A, Diomedea L, *et al.* Two amino acid substitutions within the first external loop of CCR5 induce human immunodeficiency virus-blocking antibodies in mice and chickens. *J Virol* 2008; 82: 4125-34.
- [103] Bogers WM, Bergmeier LA, Ma J, *et al.* A novel HIV-CCR5 receptor vaccine strategy in the control of mucosal SIV/HIV infection. *Aids* 2004; 18: 25-36.
- [104] Misumi S, Nakayama D, Kusaba M, *et al.* Effects of immunization with CCR5-based cycloimmunogen on simian/HIVSF162P3 challenge. *J Immunol* 2006; 176: 463-71.
- [105] Pugach P, Ketas TJ, Michael E, Moore JP. Neutralizing antibody and anti-retroviral drug sensitivities of HIV-1 isolates resistant to small molecule CCR5 inhibitors. *Virology* 2008; 377: 401-7.
- [106] Gaertner H, Cerini F, Escola JM, *et al.* Highly potent, fully recombinant anti-HIV chemokines: reengineering a low-cost microbicide. *Proc Natl Acad Sci USA* 2008; 105: 17706-11.
- [107] Fauci A. Pathogenesis of HIV disease: opportunities for new prevention interventions. *Clin Infect Dis* 2007; 45: S206-12.
- [108] Klasse PJ, Shattock R, Moore JP. Antiretroviral drug-based microbicides to prevent HIV-1 sexual transmission. *Annu Rev Med* 2008; 59: 455-71.
- [109] Quillent C, Oberlin E, Braun J, *et al.* HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CCR5 gene. *Lancet* 1998; 351: 14-8.
- [110] Paxton WA, Kang S. Chemokine receptor allelic polymorphisms: relationships to HIV resistance and disease progression. *Semin Immunol* 1998; 10: 187-94.
- [111] Wu L, Paxton WA, Kassam N, *et al.* CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, *in vitro*. *J Exp Med* 1997; 185: 1681-91.
- [112] Telenti A. Safety concerns about CCR5 as an antiviral target. *Curr Opin HIV AIDS* 2009; 4: 131-5.
- [113] Wheeler J, McHale M, Jackson V, Penny M. Assessing theoretical risk and benefit suggested by genetic association studies of CCR5: experience in a drug development programme for maraviroc. *Antivir Ther* 2007; 12: 233-45.
- [114] Schroder C, Pierson RN, 3rd, Nguyen BN, *et al.* CCR5 blockade modulates inflammation and alloimmunity in primates. *J Immunol* 2007; 179: 2289-99.
- [115] Fatkenheuer G, Nelson M, Lazzarin A, *et al.* Subgroup analyses of maraviroc in previously treated R5 HIV-1 infection. *N Engl J Med* 2008; 359: 1442-55.

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