Minireview

The CCR5-delta32 polymorphism as a model to study host adaptation against infectious diseases and to develop new treatment strategies

Gero Hütter[1] and Susanne Ganepola[2]

[1]Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, German Red Cross Blood Service Baden-Württemberg-Hessen, Heidelberg University, 68167 Mannheim; [2]II Medical Department, Asklepios Klinik Altona, Paul-Ehrlich-Straße 1, D-22763 Hamburg, Germany
Corresponding author: Gero Hütter. Email: gero.huetter@medma.uni-heidelberg.de

Abstract

Humans respond differently toward exposure against pathogens and some individuals are completely resistant against transmission due to a genetically determined susceptibility. A rising number of such, so-called, host factors have been described during the last years, but their role for diagnostic or therapeutic application is still to be clarified. Here, we describe the biology of the chemokine receptor CCR5 and its polymorphism in the context of host adaptation and immune system function. Furthermore, the first clinical applications exploiting our knowledge of this chemokine receptor as a host factor are described.

Keywords: CCR5, polymorphism, HIV, host adaptation

Experimental Biology and Medicine 2011; **236**: 938–943. DOI: 10.1258/ebm.2011.010241

Introduction

In 1949, J B S Haldane published the speculative review 'Disease and Evolution'. Therein, he presented for the first time the thesis that host factors may play a crucial role in adaptation against infectious diseases.[1] Actually, individuals respond remarkably differently toward exposure against the same pathogens and even more, some of them are completely resistant against transmission. This can partly be explained by alterations in the genome, leading to a decreased susceptibility of transmission, or an increased ability in elimination of the pathogen. In this context, probably the best-known example of such a beneficial mutation is found in the association of malaria and the hemoglobinopathies. For instance, individuals heterozygote for the beta-globulin sickle cell anemia, display a strong protection against death and severe course of disease after infection with *Plasmodium* species. Although there are hundreds of structural variants of hemoglobin, only those coding for the malaria-resistant variants have polymorphic frequencies reaching 15–20% in some parts of sub-Saharan Africa and exceeding 60% in some south-east Asian populations. Furthermore, this mutation implies such a positive selective benefit that there is no disadvantage for maintaining a population, although homozygotes display serious consequences of this variation, including early death of the individual.[2]

In contrast, today, several genetic polymorphisms are described while the best beneficial effect is achieved if the mutation or disposition is homozygous. One of the best-investigated gene polymorphism has been found for the CC chemokine receptor 5 (CCR5). Chemokine receptors are transmembrane cell surface molecules that were originally identified in the context of leukocyte trafficking. By binding several peptides, receptor-bearing cells migrate toward tissues that secrete chemokines. CCR5 in combination with CD4 gained substantial interest as a crucial host factor for cell entry exploited by the human immunodeficiency virus type 1 (HIV-1).[3] Indeed, the interaction of CCR5 and HIV-1 does not represent the natural function of this chemokine receptor. To date, the exact physiological role of CCR5 is speculative and we have achieved insights into CCR5 function rather in individuals or cell systems where the receptor is depleted. Several mutations in the promoter region as well as a 32-base pair deletion (CCR5-delta32) in the exon of CCR5 lead to a decreased expression of up to a complete deficiency of this receptor. The high allelic frequency of CCR5-delta32 of 10–15% in Caucasians and the misbalance in geographical distribution of this mutation is suggestive to assume a beneficial role for the CCR5 depletion and confers adaptation of the host toward (unknown) environmental threats.[4]

The CCR5 chemokine receptor

Structure and function

The chemokine receptor CCR5 belongs to the superfamily of the seven-transmembrane G-protein-coupled receptors (GPCRs).[5] It interacts with chemokines that mediate the trafficking and functions of memory/effector T-lymphocytes, macrophages and immature dendritic cells toward sites of inflammation. When bound by their chemokine ligands, these receptors are internalized, impairing the subsequent ability to bind their ligands. Once internalized, these receptors tend to recycle to the cell surface in time. Most chemokines activate more than one receptor subtype and like other chemokine receptors, CCR5 can bind several chemokines.[6] After activation with small ligands, GPCRs are rapidly phosphorylated at serine and threonine residues within the C-tail and the third intracellular loop.[7]

Gene regulation

The *CCR5* gene is mapped to the short arm of chromosome 3 among a group of genes that encode multiple chemokine receptors.[8,9] *CCR5* up-regulation has been proposed by nuclear factor kappa-light-chain-enhancer (NF-κB), but recently it has been suggested that gene regulated is modified by a cyclic adenosine monophosphate (cAMP)/cAMP response element-binding protein (CREB) pathway.[10,11] A selection of previously described factors that may regulate CCR5 are given in Table 1.

Physiological function

The exact physiological function of CCR5 has been entirely unknown for a long time. Individuals lacking CCR5 display no remarkable illness and no increased susceptibility toward infectious diseases could be observed until Lim *et al.*[12] figured out a possible role for CCR5 during infection with the West Nile virus (WNV). They found an increased risk for individuals with the CCR5-delta32-mutation developing fatal encephalitis and therefore suggest that the functional receptor acts by recruiting leukocytes into the infected central nervous system. Nevertheless, CCR5 deficiency is not a risk factor for WNV infection *per se*, but is a risk factor for both early and late clinical manifestations after WNV-infection.[13]

CCR5 and HIV cell entry

HIV-1 uses the highly conserved host elements of both CD4 and CCR5 for cell entry. The HIV envelope protein is comprised of three heterodimeric glycoproteins; each consists of a transmembrane glycoprotein 41 (gp41) non-covalently associated with glycoprotein 120 (gp120). In the first step, gp120 binds to cellular CD4, causing a conformational change in the envelope glycoprotein. In the second step, the conformation change exposes previously inaccessible domains permitting binding to the CCR5 co-receptor. This new association provides a second confirmation change in the third step as it induces the uncovering of the free amino terminal-fusion domain of gp41 consequently embedded into the host cell membrane, to link the viral and host cell membrane. The fusion of virion and host cell is mediated by forming a 6-helix bundle of gp41 bringing the viral membrane and host cell membrane together.[14]

Table 1 Regulation of CCR5 expression

Factor	Effect on CCR5
IFN-alpha[55]	Up-regulation
cAMP/CREB pathway[10]	Up-regulation
IL-15[56]	Up-regulation
IL-4 together with IL-10[57]	Up-regulation
IL-1β[58]	Down-regulation

IFN, interferon; IL, interleukin; cAMP, cyclic adenosine monophasphate; CREB, cAMP response element-binding protein

CCR5 polymorphism

Soon after the detection of CCR5 as a co-factor for HIV-1 cell entry, several mutations in this gene have been found. To date, there are at least 74 mutations described.[15–17] Several studies have demonstrated that polymorphism at the *CCR5* locus both in the coding and the regulatory regions may affect susceptibility toward HIV infection. The much-studied *CCR5*-delta*32* allele is a 32-base pair deletion that introduces a premature stop-codon into the *CCR5* chemokine-receptor locus and thus obliterates the receptor.[18] Epidemiological studies of the Caucasian population demonstrated that the CCR5-delta32 deletion shows the highest frequency of 10–20% among the heterozygous, and 1% among the homozygous karyotype. Interestingly, the deletion cannot be found in the Asian, Middle East, African and the American Indian population.[19] It is supposed that the imbalanced distribution of this allele is caused by environmental-based selective pressure, resulting in a privilege for the delta32 deletion.[4,20]

Suggested candidates for this positive selection pressure include protection from plague caused by *Yersinia pestis*, smallpox or other related poxviruses. Nevertheless, there is no laboratory evidence for this thesis in addition to the unrevealed physiological role of this chemokine receptor.[21–23]

Consequences of CCR5 polymorphism

CCR5-delta32 and HIV infection

Homozygosity for CCR5-delta32 deletion is associated with a high, but not complete, protection against HIV-1.[8,24] Although CCR5-delta32 results in a high degree of resistance toward HIV-1-transmission, several individuals tested to be homozygous for this mutation acquired HIV-infection.[25–28] Under certain circumstances, HIV-1 quasispecies are able to use CXCR4 as an alternative co-receptor instead of CCR5. The responsible mutation occurs at V3 of the HIV-1 genome encoding an envelope glycoprotein (gp120) binding either to CCR5 (R5 type) or CXCR4 (X4 type).[29] The switch from R5 to X4 occurs in the natural course of infection and the rate of X4 increases to approximately 50% during antiretroviral therapy.[30] A trial with 979 HIV+ and antiretroviral-naïve individuals demonstrated that 18.2% were harboring an X4-variant of HIV-1.[31]

Interestingly, although several individuals were frequently exposed to X4, the R5 variant dominates during transmission and early infection, irrespective of the course of transmission.[32] Furthermore, despite the fact that CCR5-delta32 homozygous individuals can be infected with X4 or R5X4 quasispecies, this practically never happens.[33] A 'gatekeeper' function preferring CCR5 using R5 is postulated during first infection showing an almost perfect negative selection of X4.[34]

Recently, we performed the successful stem cell transplantation (SCT) of an HIV-1-infected patient suffering from acute myeloid leukemia, applying homozygous *CCR5*-delta*32* donor cells. These hematopoietic progenitor cells engrafted, proliferated and differentiated to produce mature myeloid and lymphoid cells that were highly resistant to HIV-1 infection. Currently, nearly four years after the allogeneic SCT and in the absence of any antiretroviral treatment, no plasma viral load or proviral DNA was detectable in peripheral blood cells or certain tissue samples, including rectal mucosa and brain tissue.[35–37]

Chronic viral infection

Murine models with CCR5 deficiency mice have demonstrated a robust T-cell response to several infectious agents.[38] A vigorous T-cell response is required to recover from acute hepatitis B virus (HBV) infection. Interestingly, Thio *et al.*[39] found that CCR5-delta32 increases the likelihood of recovery form HBV infection and reduces the development of chronic HBV infection by nearly 50%. Furthermore, this protective effect was exclusively mediated by the CCR5-delta32 deletion and not by any of the other neighboring polymorphisms.

Allogeneic SCT and graft versus host disease

Chemokines play a crucial role in the pathogenesis of graft versus host disease (GvHD) disease after allogeneic SCT. In experimental models, due to the redundancy of receptor − ligand interaction, the deficiency or blockade of a single chemokine does not protect the allograft from acute rejection.[40] However, recent studies have demonstrated that the blockade or absence of a single chemokine receptor does prolong allograft survival in a fully major histocompatibility complex (MHC) mismatched model.[41]

In a study with CCR5-knockout and wild-type mice, animals were lethally irradiated and underwent full MHC-mismatch bone marrow transplantation. Observing more cases of GvHD in the group of CCR5-knockout mice, Kuziel and colleagues[42] concluded that the absence of CCR5 results in donor T-cell expansion with a consecutive higher rate of GvHD. In another animal model, the authors demonstrated that CCR5 and CXCR3 combined chemokine blockade is effective in prolonging allograft survival and limiting acute rejection concurrently.[43]

A study investigating the CCR5 polymorphism from donors of 186 allografted recipients demonstrated contradicting results to those of Kuziel. Here, the authors suggested that the presence of the CCR5-delta32 allele represents a protective factor regarding the risk of developing GvHD after allogeneic SCT.[44] Previously, Murai *et al.*[45] described the recruitment of CCR5-expression CD8+ T-cells during acute liver GvHD in patients after allogeneic SCT.

Most recently, a significant association of the common CCR5 haplotype (H1/H1) and advantage of disease-free survival and overall survival in recipients of allogeneic SCT has been found. The authors suggested CCR5 genotyping as a new diagnostic and therapeutic strategy for therapy optimization.[46]

Organ transplantation and graft rejection

Recipients of organ allografts homozygous for CCR5-delta32 show longer survival of transplant function than those with other genotypes. This has been shown for renal and liver transplants suggesting that patients with CCR5-delta32 might be candidates for a reduced immunosuppressive therapy.[40,47] Furthermore, interaction and blockade of the CCR5 receptor may also reduce alloantigen-specific T-lymphocyte proliferation and may be effective in preventing acute and chronic rejection of allograft.[48]

CCR5-targeted therapy

Based on the challenges of the pharmacological interaction with CCR5 during HIV-1 therapy, we have gained deep insights into the action of CCR5 blocking agents.[49] These experiences can also be useful for other applications besides HIV-1 infection, for example, transplantation medicine. Interference with CCR5 function or expression in terms of manipulation of the immune system and responses seems to be a promising approach. Both CCR5 blockade and down-regulation with rapamycin are effective in modulating transplant immunity and lead to prolonged allograft survival in the animal model.[50]

Currently, two clinical trials are investigating the effect of interaction with the CCR5 receptor system in patients with rheumatoid arthritis and patients following allogeneic SCT (NHI ClinicalTrials.gov Identifier: NCT00948753 & NCT00979771), but supposable applications of this approach are numerous.[51]

Conclusions

Taken into account the actual technology in comparison with earlier times, we are now able to identify host factors in a more sufficient way. Additionally, new biotechnical methodology has improved our ability to generate a patient-optimized therapy based on individual host factors. This enables us to predict, prevent, diagnose and treat subtypes of diseases in a more efficient way.[52] Our diagnostic and therapeutic tools in individualized medicine have rapidly advanced in the last few years, such as the automated Sanger method, which has been considered as a 'first-generation' technology, and even more recent methods are referred to as next-generation sequencing. These new technologies include a mixture of several techniques: bacterial cloning or polymerase chain reaction, template purification, labeling of DNA fragments using the chain termination

Table 2 Host factors and infectious diseases

Host gene	Agent	Mechanism
HB α-globin[59]	*Plasmodium*	Polymerization of hemoglobin
HB β-globin[60]	*Plasmodium*	Polymerization of hemoglobin
G6PD[61]	*Plasmodium*	Pentose phosphate pathway
HLA B53[62]	*Plasmodium*	Presents antigens to T-cells
Duffy antigen[63]	*Plasmodium*	Chemokine receptor
CCR5[18]	HIV-1	Cell entry
CCR2[64]	HIV-1	Cell entry
HLA DRB11302*[65]	Hepatitis B virus	Presents antigens to T-cells
HLA DRB11101*[66]	Hepatitis C virus	Presents antigens to T-cells
IL-28B[67]	Hepatitis C virus	Induction of viral defense
Tetherin/ BST-2[68]	Lentivirus	Restricts virion release
CISH*[69]	Bacteremia, malaria and tuberculosis	Suppressor of cytokine signaling
Toll-like receptor 2[70]	*Chlamydia trachomatis*	Pathogen recognition
Toll-like receptor 3[71,72]	WNV, Influenza virus	Rrecognizes double-stranded RNA
OAS1[73]	SARS-associated coronavirus	Activates latent RNase

*Cytokine-inducible Src homology 2 (SH2) domain protein
SARS, severe acute respiratory syndrome

method with energy transfer, dye-labeled dideoxynucleotides and a DNA polymerase, capillary electrophoresis and fluorescence detection providing four-color plots to reveal the DNA sequence.[53] Most recently, the 454 Sequencing, as a next-generation sequencing technology, features a unique mix of long reads, exceptional accuracy and ultra-high throughput.[54]

Studying host factors and their genetic contribution to infectious and other diseases presumes fundamental understanding of pathogenesis (Table 2). The association of candidate gene polymorphisms in several circumstances, not only for infectious diseases, has provided new strategies of intervention. In the future, genomic testing will allow us to perform both prognostic and predictive sub-typing of patient populations. Patients may benefit from therapeutics targeted to their specific disease processes and will (probably) therefore have a reduced risk regarding the development of life-threatening adverse events caused, for example, by genetic differences in drug metabolism. Finally, this kind of revolution in regenerative medicine and therapeutics offers the possibility to transform the efficiency of managing disease from palliation up to cure.

Author contributions: GH and SG wrote the manuscript.

ACKNOWLEDGEMENTS

We thank Kate Krauss and Stephen LeBlanc from the AIDS Policy Project for supporting our work.

REFERENCES

1 Lederberg J. JBS Haldane (1949) on infectious disease and evolution. *Genetics* 1999;**153**:1–3
2 Min-Oo G, Gros P. Erythrocyte variants and the nature of their malaria protective effect. *Cell Microbiol* 2005;**7**:753–63
3 Oppermann M. Chemokine receptor CCR5: insights into structure, function, and regulation. *Cell Signal* 2004;**16**:1201–10
4 Galvani AP, Novembre J. The evolutionary history of the CCR5-Delta32 HIV-resistance mutation. *Microbes Infect* 2005;**7**:302–9
5 Allen SJ, Crown SE, Handel TM. Chemokine: receptor structure, interactions, and antagonism. *Annu Rev Immunol* 2007;**25**:787–820
6 Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000;**18**:217–42
7 Marchese A, Paing MM, Temple BR, Trejo J. G protein-coupled receptor sorting to endosomes and lysosomes. *Annu Rev Pharmacol Toxicol* 2008;**48**:601–29
8 Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C, Muyldermans G, Verhofstede C, Burtonboy G, Georges M, Imai T, Rana S, Yi Y, Smyth RJ, Collman RG, Doms RW, Vassart G, Parmentier M. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 1996;**382**:722–5
9 Hütter G, Neumann M, Nowak D, Klüter H, Hofmann WK. The beneficial effect of the CCR5-d32 deletion in GvHD. Chemokine function or genecluster coexpression aside HLA match? *Vox Sanguinis* 2010;**99**(S1):P-1101
10 Kuipers HF, Biesta PJ, Montagne LJ, van Haastert ES, van der Valk P, van den Elsen PJ. CC chemokine receptor 5 gene promoter activation by the cyclic AMP response element binding transcription factor. *Blood* 2008;**112**:1610–9
11 Liu R, Zhao X, Gurney TA, Landau NR. Functional analysis of the proximal CCR5 promoter. *AIDS Res Hum Retroviruses* 1998;**14**:1509–19
12 Lim JK, Glass WG, McDermott DH, Murphy PM. CCR5: no longer a 'good for nothing' gene – chemokine control of West Nile virus infection. *Trends Immunol* 2006;**27**:308–12
13 Lim JK, McDermott DH, Lisco A, Foster GA, Krysztof D, Follmann D, Stramer SL, Murphy PM. CCR5 deficiency is a risk factor for early clinical manifestations of West Nile virus infection but not for viral transmission. *J Infect Dis* 2010;**201**:178–85
14 Lederman MM, Penn-Nicholson A, Cho M, Mosier D. Biology of CCR5 and its role in HIV infection and treatment. *JAMA* 2006;**296**:815–26
15 Bream JH, Young HA, Rice N, Martin MP, Smith MW, Carrington M, O'Brien SJ. CCR5 promoter alleles and specific DNA binding factors. *Science* 1999;**284**:223
16 Carrington M, Dean M, Martin MP, O'Brien SJ. Genetics of HIV-1 infection: chemokine receptor CCR5 polymorphism and its consequences. *Hum Mol Genet* 1999;**8**:1939–45
17 Ensemble. See http://www.ensembl.org (last checked 26 March 2011)
18 Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 1996;**86**:367–77
19 Stephens JC, Reich DE, Goldstein DB, Shin HD, Smith MW, Carrington M, Winkler C, Huttley GA, Allikmets R, Schriml L, Gerrard B, Malasky M, Ramos MD, Morlot S, Tzetis M, Oddoux C, di Giovine FS, Nasioulas G, Chandler D, Aseev M, Hanson M, Kalaydjieva L, Glavac D, Gasparini P, Kanavakis E, Claustres M, Kambouris M, Ostrer H, Duff G, Baranov V, Sibul H, Metspalu A, Goldman D, Martin N, Duffy D, Schmidtke J, Estivill X, O'Brien SJ, Dean M. Dating the origin of the CCR5-Delta32 AIDS-resistance allele by the coalescence of haplotypes. *Am J Hum Genet* 1998;**62**:1507–15
20 Libert F, Cochaux P, Beckman G, Samson M, Aksenova M, Cao A, Czeizel A, Claustres M, de la Rua C, Ferrari M, Ferrec C, Glover G, Grinde B, Guran S, Kucinskas V, Lavinha J, Mercier B, Ogur G, Peltonen L, Rosatelli C, Schwartz M, Spitsyn V, Timar L, Beckman L, Parmentier M, Vassart G. The deltaccr5 mutation conferring protection against HIV-1 in Caucasian populations has a single and recent origin in Northeastern Europe. *Hum Mol Genet* 1998;**7**:399–406
21 Elvin SJ, Williamson ED, Scott JC, Smith JN, Perez De Lema G, Chilla S, Clapham P, Pfeffer K, Schlondorff D, Luckow B. Evolutionary genetics: ambiguous role of CCR5 in *Y. pestis* infection. *Nature* 2004;**430**:417

22 Mecsas J, Franklin G, Kuziel WA, Brubaker RR, Falkow S, Mosier DE. Evolutionary genetics: CCR5 mutation and plague protection. *Nature* 2004;**427**:606

23 Lalani AS, Masters J, Zeng W, Barrett J, Pannu R, Everett H, Arendt CW, McFadden G. Use of chemokine receptors by poxviruses. *Science* 1999;**286**:1968–71

24 Sheppard HW, Celum C, Michael NL, O'Brien S, Dean M, Carrington M, Dondero D, Buchbinder SP. HIV-1 infection in individuals with the CCR5-Delta32/Delta32 genotype: acquisition of syncytium-inducing virus at seroconversion. *J Acquir Immune Defic Syndr* 2002;**29**:307–13

25 Biti R, Ffrench R, Young J, Bennetts B, Stewart G, Liang T. HIV-1 infection in an individual homozygous for the CCR5 deletion allele. *Nat Med* 1997;**3**:252–3

26 Theodorou I, Meyer L, Magierowska M, Katlama C, Rouzioux C. HIV-1 infection in an individual homozygous for CCR5 delta 32. Seroco Study Group. *Lancet* 1997;**349**:1219–20

27 Michael NL, Nelson JA, KewalRamani VN, Chang G, O'Brien SJ, Mascola JR, Volsky B, Louder M, White GC 2nd, Littman DR, Swanstrom R, O'Brien TR. Exclusive and persistent use of the entry coreceptor CXCR4 by human immunodeficiency virus type 1 from a subject homozygous for CCR5 delta32. *J Virol* 1998;**72**:6040–7

28 Gorry PR, Zhang C, Wu S, Kunstman K, Trachtenberg E, Phair J, Wolinsky S, Gabuzda D. Persistence of dual-tropic HIV-1 in an individual homozygous for the CCR5 Delta 32 allele. *Lancet* 2002;**359**:1832–4

29 Rosen O, Sharon M, Quadt-Akabayov SR, Anglister J. Molecular switch for alternative conformations of the HIV-1 V3 region: implications for phenotype conversion. *Proc Natl Acad Sci U S A* 2006;**103**:13950–5

30 Delobel P, Sandres-Saune K, Cazabat M, Pasquier C, Marchou B, Massip P, Izopet J. R5 to X4 switch of the predominant HIV-1 population in cellular reservoirs during effective highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2005;**38**:382–92

31 Brumme ZL, Goodrich J, Mayer HB, Brumme CJ, Henrick BM, Wynhoven B, Asselin JJ, Cheung PK, Hogg RS, Montaner JS, Harrigan PR. Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. *J Infect Dis* 2005;**192**:466–74

32 van't Wout AB, Kootstra NA, Mulder-Kampinga GA, Albrecht-van Lent N, Scherpbier HJ, Veenstra J, Boer K, Coutinho RA, Miedema F, Schuitemaker H. Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission. *J Clin Invest* 1994;**94**:2060–7

33 Gray L, Churchill MJ, Keane N, Sterjovski J, Ellett AM, Purcell DF, Poumbourios P, Kol C, Wang B, Saksena NK, Wesselingh SL, Price P, French M, Gabuzda D, Gorry PR. Genetic and functional analysis of R5X4 human immunodeficiency virus type 1 envelope glycoproteins derived from two individuals homozygous for the CCR5delta32 allele. *J Virol* 2006;**80**:3684–91

34 Margolis L, Shattock R. Selective transmission of CCR5-utilizing HIV-1: the 'gatekeeper' problem resolved? *Nat Rev Microbiol* 2006;**4**:312–7

35 Hütter G, Nowak D, Mossner M, Ganepola S, Mussig A, Allers K, Schneider T, Hofmann J, Kucherer C, Blau O, Blau IW, Hofmann WK, Thiel E. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 2009;**360**:692–8

36 Hütter G, Schneider T, Thiel E. Transplantation of selected or transgenic blood stem cells – a future treatment for HIV/AIDS? *J Int AIDS Soc* 2009;**12**:10

37 Allers K, Hutter G, Hofmann J, Loddenkemper C, Rieger K, Thiel E, Schneider T. Evidence for the cure of HIV infection by CCR5{Delta}32/{Delta}32 stem cell transplantation. *Blood* 2010;**117**:2791–9

38 Algood HM, Flynn JL. CCR5-deficient mice control Mycobacterium tuberculosis infection despite increased pulmonary lymphocytic infiltration. *J Immunol* 2004;**173**:3287–96

39 Thio CL, Astemborski J, Bashirova A, Mosbruger T, Greer S, Witt MD, Goedert JJ, Hilgartner M, Majeske A, O'Brien SJ, Thomas DL, Carrington M. Genetic protection against hepatitis B virus conferred by CCR5Delta32: Evidence that CCR5 contributes to viral persistence. *J Virol* 2007;**81**: 441–5

40 Fischereder M, Luckow B, Hocher B, Wuthrich RP, Rothenpieler U, Schneeberger H, Panzer U, Stahl RA, Hauser IA, Budde K, Neumayer H, Kramer BK, Land W, Schlondorff D. CC chemokine receptor 5 and renal-transplant survival. *Lancet* 2001;**357**:1758–61

41 Gao W, Faia KL, Csizmadia V, Smiley ST, Soler D, King JA, Danoff TM, Hancock WW. Beneficial effects of targeting CCR5 in allograft recipients. *Transplantation* 2001;**72**:1199–205

42 Welniak LA, Wang Z, Sun K, Kuziel W, Anver MR, Blazar BR, Murphy WJ. An absence of CCR5 on donor cells results in acceleration of acute graft-versus-host disease. *Exp Hematol* 2004;**32**:318–24

43 Schnickel GT, Hsieh GR, Garcia C, Shefizadeh A, Fishbein MC, Ardehali A. Role of CXCR3 and CCR5 in allograft rejection. *Transplant Proc* 2006;**38**:3221–4

44 Bogunia-Kubik K, Duda D, Suchnicki K, Lange A. CCR5 deletion mutation and its association with the risk of developing acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Haematologica* 2006;**91**:1628–34

45 Murai M, Yoneyama H, Harada A, Yi Z, Vestergaard C, Guo B, Suzuki K, Asakura H, Matsushima K. Active participation of CCR5(+)CD8(+) T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. *J Clin Invest* 1999;**104**:49–57

46 McDermott DH, Conway SE, Wang T, Ricklefs SM, Agovi MA, Porcella SF, Tran HT, Milford E, Spellman S, Abdi R. Donor and recipient chemokine receptor CCR5 genotype is associated with survival after bone marrow transplantation. *Blood* 2010;**115**:2311–8

47 Heidenhain C, Puhl G, Moench C, Lautem A, Neuhaus P. Chemokine receptor 5Delta32 mutation reduces the risk of acute rejection in liver transplantation. *Ann Transplant* 2009;**14**:36–44

48 Schnickel GT, Bastani S, Hsieh GR, Shefizadeh A, Bhatia R, Fishbein MC, Belperio J, Ardehali A. Combined CXCR3/CCR5 blockade attenuates acute and chronic rejection. *J Immunol* 2008;**180**:4714–21

49 Fatkenheuer G, Pozniak AL, Johnson MA, Plettenberg A, Staszewski S, Hoepelman AI, Saag MS, Goebel FD, Rockstroh JK, Dezube BJ, Jenkins TM, Medhurst C, Sullivan JF, Ridgway C, Abel S, James IT, Youle M, van der Ryst E. Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat Med* 2005;**11**:1170–2

50 Li J, Zhang K, Ye P, Wang S, Xia J. CCR5 blockade in combination with rapamycin prolongs cardiac allograft survival in mice. *Clin Exp Immunol* 2009;**157**:437–45

51 Hütter G, Allers K, Schneider T. The additional use of viral entry inhibitors during autologous hematopoietic stem cell transplantation in patients with non-Hodgkin's lymphoma and HIV-1 infection. *Biol Blood Marrow Transplant* 2011;**17**:586–7

52 Cortese DA. A vision of individualized medicine in the context of global health. *Clin Pharmacol Ther* 2007;**82**:491–3

53 Metzker ML. Sequencing technologies – the next generation. *Nat Rev Genet* 2010;**11**:31–46

54 Droege M, Hill B. The Genome Sequencer FLX System–longer reads, more applications, straight forward bioinformatics and more complete data sets. *J Biotechnol* 2008;**136**:3–10

55 Stoddart CA, Keir ME, McCune JM. IFN-alpha-induced upregulation of CCR5 leads to expanded HIV tropism in vivo. *PLoS Pathog* 2010;**6**:e1000766

56 Wang CR, Liu MF. Regulation of CCR5 expression and MIP-1alpha production in CD4+ T cells from patients with rheumatoid arthritis. *Clin Exp Immunol* 2003;**132**:371–8

57 Jiang PJ, Zhao AM, Bao SM, Xiao SJ, Xiong M. Expression of chemokine receptors CCR3, CCR5 and CXCR3 on CD4(+) T cells in CBA/JxDBA/2 mouse model, selectively induced by IL-4 and IL-10, regulates the embryo resorption rate. *Chin Med J (Engl)* 2009;**122**:1917–21

58 Vassiliadis S, Balabanidou V, Papadopoulos GK, Athanassakis I. Localization and expression of CCR3 and CCR5 by interleukin-1 beta in the RIN-5AH insulin-producing model system: a protective mechanism involving down-regulation of chemokine receptors. *JOP* 2002;**3**:66–75

59 Allen SJ, O'Donnell A, Alexander ND, Alpers MP, Peto TE, Clegg JB, Weatherall DJ. alpha+-Thalassemia protects children against disease caused by other infections as well as malaria. *Proc Natl Acad Sci U S A* 1997;**94**:14736–41

60 Willcox M, Bjorkman A, Brohult J. Falciparum malaria and beta-thalassaemia trait in northern Liberia. *Ann Trop Med Parasitol* 1983;**77**:335–47

61 Ruwende C, Khoo SC, Snow RW, Yates SN, Kwiatkowski D, Gupta S, Warn P, Allsopp CE, Gilbert SC, Peschu N, Newbold CI, Greenwood BM, Marsh K, Hill AVS. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. *Nature* 1995;**376**:246–9

62 Hill AV, Allsopp CE, Kwiatkowski D, Anstey NM, Twumasi P, Rowe PA, Bennett S, Brewster D, McMichael AJ, Greenwood BM. Common west African HLA antigens are associated with protection from severe malaria. *Nature* 1991;**352**:595–600
63 Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria. *Clin Microbiol Rev* 2002;**15**:564–94
64 Chatterjee K. Host genetic factors in susceptibility to HIV-1 infection and progression to AIDS. *J Genet* 2010;**89**:109–16
65 Thursz MR, Kwiatkowski D, Allsopp CE, Greenwood BM, Thomas HC, Hill AV. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. *N Engl J Med* 1995;**332**:1065–9
66 Thursz M, Yallop R, Goldin R, Trepo C, Thomas HC. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. *Lancet* 1999;**354**:2119–24
67 Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009;**461**:798–801
68 Lim ES, Malik HS, Emerman M. Ancient adaptive evolution of tetherin shaped the functions of vpu and nef in human immunodeficiency virus and primate lentiviruses. *J Virol* 2010;**84**:7124–34
69 Khor CC, Vannberg FO, Chapman SJ, Guo H, Wong SH, Walley AJ, Vukcevic D, Rautanen A, Mills TC, Chang KC, Kam KM, Crampin AC, Ngwira B, Leung CC, Tam CM, Chan CY, Sung JJ, Yew WW, Toh KY, Tay SK, Kwiatkowski D, Lienhardt C, Hien TT, Day NP, Peshu N, Marsh K, Maitland K, Scott JA, Williams TN, Berkley JA, Floyd S, Tang NL, Fine PE, Goh DL, Hill AV. CISH and susceptibility to infectious diseases. *N Engl J Med* 2010;**362**:2092–101
70 Karimi O, Ouburg S, de Vries HJ, Pena AS, Pleijster J, Land JA, Morre SA. TLR2 haplotypes in the susceptibility to and severity of Chlamydia trachomatis infections in Dutch women. *Drugs Today (Barc)* 2009;**45** (Suppl B):67–74
71 Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat Med* 2004;**10**:1366–73
72 Le Goffic R, Balloy V, Lagranderie M, Alexopoulou L, Escriou N, Flavell R, Chignard M, Si-Tahar M. Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathog* 2006;**2**:e53
73 He J, Feng D, de Vlas SJ, Wang H, Fontanet A, Zhang P, Plancoulaine S, Tang F, Zhan L, Yang H, Wang T, Richardus JH, Habbema JD, Cao W. Association of SARS susceptibility with single nucleic acid polymorphisms of OAS1 and MxA genes: a case-control study. *BMC Infect Dis* 2006;**6**:106