Correspondence

AIDS 2007, **21**:111–122

A novel 24-base pair deletion in the coding region of CCR5 in an African population

The chemokine receptor CCR5 is the main co-receptor for macrophage-tropic HIV-1 strains, which are responsible for virus transmission and predominate during the asymptomatic phase of the disease [1]. This has been demonstrated by the almost complete resistance of individuals homozygous for a 32-base pair (bp) deletion in the coding region of the receptor (CCR5Δ32) [2]. Other natural genetic variants of the CCR5 coding region or promoter have been described that modulate HIV-1 disease progression or the function of the receptor [3].

During a study analysing the influence of genetic host factors on the risk of vertical transmission of HIV-1 in Kigali, Rwanda, we identified the heterozygous carriage of a novel 24-bp deletion in the coding region of CCR5 (denoted hCCR5Δ24) in an infected mother and her infected child. In addition, we detected two subjects heterozygous for hCCR5Δ24 in a cohort of 36 HIV-1-serodiscordant couples originating from the same region.

A total of 178 pairs of HIV-1-positive women and children in Kigali were enrolled in a cohort study for the prevention of mother-to-child HIV-1 transmission, from March 2002 to October 2004. Frozen peripheral blood mononuclear cell pellets were collected and sent to the Retrovirology Laboratory in Luxembourg for the analysis of HIV co-receptor polymorphisms, including a stop codon at amino acid position 101 of CCR5 (mutation m303 [4]). For the sequencing of samples encoding hCCR5Δ24, DNA extracts were amplified using a nested polymerase chain reaction (PCR) protocol. The outer PCR was performed using primers 326F (5′–TGCTTGGCCAAAAAGAGAGT–3′) and 977R (5′–GATGATTCCTGGGAGAGACG–3′) and was subsequently separated on a 2% agarose gel electrophoresis. The 24-bp-deleted fragments were gel-purified using the QiaexII kit (Qiagen, Venlo, The Netherlands) and an inner PCR was performed using primers 472F (5′–GGTGGAACAAGATGGATTATCAAGAGT–3′) and 882R (5′–CAGCATGGACGACAGCCAGG–3′). Sequencing was performed on purified amplicons using the BigDye terminator cycle-sequencing chemistry (PE Applied Biosystems, Foster City, California, USA), and primers 472F and 882R.

Although the screening for the m303 mutation proved negative for all women and children, we identified an unusual migration pattern on the gel electrophoresis in an infected mother and her infected child, suggesting the heterozygous carriage of a large deletion (Fig. 1a). The screening was extended to a cohort of 36 serodiscordant couples, and revealed the existence of two unrelated heterozygotes for hCCR5Δ24, one infected and one non-infected. Compared with a reference (GenBank accession no. U95626), the sequence analysis revealed a 24-bp deletion from nucleotide 61730 to 61753 (accession no. AY947540), involving eight amino acids (V_{83}–A_{90}) located in the second transmembrane helix (Fig. 1b and c).

To date, the most widely studied CCR5 mutation is CCR5Δ32, which results in a non-functional protein. The prevalence of CCR5Δ32 is extremely low in sub-Saharan Africa [5,6], so the presence of the 24-bp deletion in a central African population is remarkable. On the basis of our observation, the allelic frequency of hCCR5Δ24 seems rare in Rwanda. However, our study on the genetic background of CCR5 in Rwanda was restricted to a selected population. A better evaluation of the prevalence of hCCR5Δ24 in Rwanda, and thus of its impact on HIV-1 transmission and disease progression, should also rely on other subject categories, for example healthy uninfected volunteers and larger groups of high-risk HIV-1-seronegative individuals.

hCCR5Δ24 is expected to affect the receptor topology and its functionality as a chemokine receptor or as an HIV-1 co-receptor, although it does not alter the reading frame of CCR5. P_{84}, F_{85} and Y_{89} are critical residues for chemokine binding affinity and the functional activation of CCR5 [7,8]. Impaired HIV-1 co-receptor function is also expected, through structural constraints in domains critical for binding the gp120 envelope subunit of R5-tropic HIV-1 and for HIV-1 fusion [9–11], or through altered CCR5 expression. Interestingly, a 24-bp deletion has been found in the CCR5 of an African simian species (red-capped mangabeys) [12]. That mutation (Δ24 CCR5) encompassed eight amino acids in frame in the fourth transmembrane helix. Red-capped mangabeys homozygous for Δ24 CCR5 were found to be naturally infected with divergent SIV, which was unable to use CCR5, but instead used CCR2b as its major co-receptor. Taken together, these findings support the likelihood that hCCR5Δ24 will impair the physiological or HIV-related functions of CCR5.

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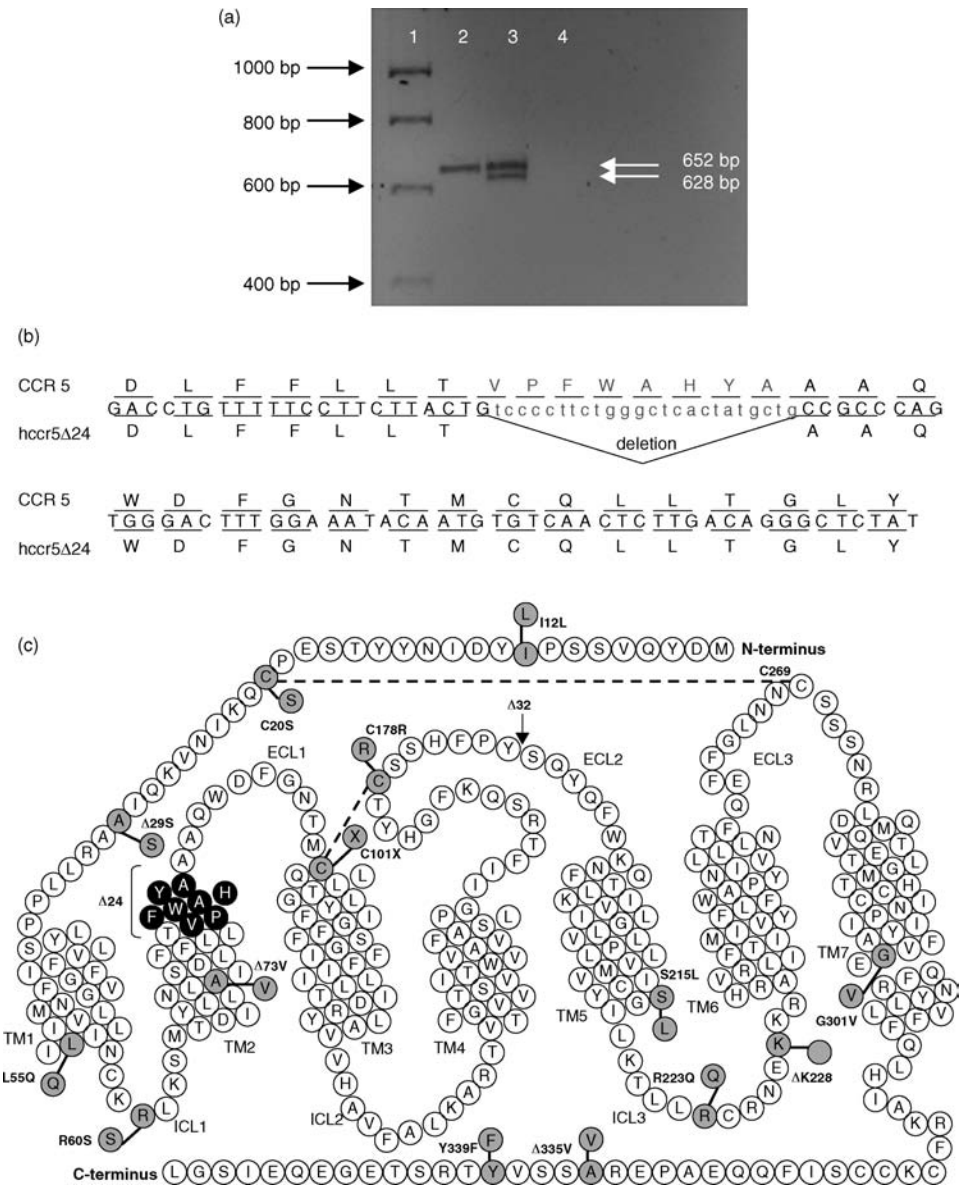

Fig. 1. Mutational analysis of hCCR5Δ24. (a) Analysis of CCR5 polymerace chain reaction fragments on a 3% agarose gel: 1. Smart ladder (Eurogentec, Seraing, Belgium); 2. CCR5WT/CCR5WT; 3. CCR5WT/hCCR5Δ24; 4. negative control. (b) Nucleotide sequence of CCR5 surrounding the deleted region, and translation into the normal receptor (top) or the truncated mutant (bottom). (c) Schematic representation of the CCR5 sequence: ECL, extracellular loop; ICL, intracellular loop; TM, transmembrane helix. Black circles denote deleted residues in hCCR5Δ24. The arrow indicates the location of CCR5Δ32 and grey-shaded circles denote other known genetic variants of CCR5 [9]. Broken lines depict disulphide bonds linking together CCR5 extracellular domains (C20–C269 and C101–C178).

Cecile Masquelier[a], Jean-Yeves Servais[a], Emmanual Rusanganwa[b], Francois Roman[a], Emmanuel Havuga[b,c], Jean Servais[a], Samuel Tuyizere[b], Christine Omes[b,c], Jean-Claude Karasi[b,c], Olivier Coruteille[b,c], Etienne Karita[d], Susan Allen[d], Jean-Claude Schmit[a] and Vic Arendt[a,b], *[a]Retrovirology Laboratory, Centre de Recherche Public (CRP)-Santé, Luxembourgh, [b]National Reference Laboratory, [c]RWA/021 TRAC LuxDevelopement, and [d]Project San Francisco, Kigali, Rwanda.*

Sponsorship: This study was supported by the 'Fondation Recherche sur le SIDA', the 'Centre de Recherche Public-Santé (CRP-Santé)' (CRP project C02/01), Lux-Development and the Ministry of Foreign Affairs, Luxembourg.

Received: 25 May 2006; accepted: 24 June 2006.

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Fatal hepatorenal failure and thrombocytopenia with SU5416, a vascular endothelial growth factor Flk-1 receptor inhibitor, in AIDS–Kaposi's sarcoma

The endothelial proliferation of Kaposi's sarcoma (KS) is dependent on vascular endothelial growth factor (VEGF). Its receptor, Flk-1/KDR, is overexpressed and active in an autocrine feedback loop [1]. SU5416, a potent inhibitor of VEGF-mediated Flk-1 receptor signaling, was tested in patients with a variety of malignancies. As angiogenesis is rarely found in adult life, inhibition was anticipated to have little toxicity. Despite a favorable toxicity profile in earlier trials with nearly 150 patients and no dose-limiting toxicity in a phase I trial in AIDS–KS (S. Miles, personal communication), the investigation of SU5416 in patients with AIDS–KS was halted after two patients died after developing thrombocytopenia, renal failure and acidosis.

Patients with biopsy-confirmed, chemotherapy-refractory or intolerant AIDS–KS, with measurable cutaneous lesions and adequate hematological, renal and hepatic function were treated in two parallel studies, one conducted in the United States by the AIDS Malignancy Consortium and one in Europe. Eligible patients received SU5416 145 mg/m^2 twice weekly intravenously.

Case 1

A 58-year-old man with AIDS–KS diagnosed in February 1999, treated with liposomal doxorubicin (17 June to 1 September 1999), received SU5416 from 5 February to 22 May 2000. Baseline platelet counts and renal function were normal, the HIV-RNA level was less than 20 copies/ml and the CD4 cell count was 388 cells/μl. Concomitant medications are shown in Table 1. On 22 May 2000, the patient presented with fatigue, fever, and leg edema. He had ascites, thrombocytopenia (29 000/μl) without schistocytes, creatinine 1.7 mg/dl, uric acid 14 mg/dl, total bilirubin 1.3 mg/dl, alkaline phosphatase 300 μ/l, gamma transferase 85 μ/l. He underwent hemodialysis and infusion of fresh frozen plasma. On 5 June 2000, the HIV-RNA level was 50 000 copies/ml and CD4 cell count was 109 cells/μl. The patient was taken off antiretroviral therapy and started on ciprofloxacin and acyclovir on 6 June 2000. The patient died on 12 June 2000.

Case 2

A 48-year-old man was diagnosed with AIDS in September 1989 and Kaposi's sarcoma in July 1999, had been treated previously with liposomal doxorubicin (24 September 1999 to 14 January 2000) and paclitaxel (14 February to 19 May 2000), and had received a single dose of SU5416 on 23 June 2000. Concomitant medications are shown in Table 1. Baseline laboratory

Table 1. Concomitant medications.

Case 1	Lamivudine, saquinavir, ritonavir, and stavudine, trimethoprim/sulfamethoxazole (PCP prophylaxis), and allopurinol 100 mg daily
Case 2	Ritonavir, amprenavir, saquinavir, stavudine, efavirenz, abacavir, lamivudine, trimethoprim/sulfamethoxazole (PCP prophylaxis), celecoxib, primaquine, loperamide, azithromycin, clotrimazole, clindamycin, calcium salts, furosemide, bumetanide, phytonadione (vitamin K), acetaminophen, diphenhydramine, prochlorperazine, hydromorphone, simethicone, lorazepam, and sodium polystyrene sulfonate.

PCP, Pneumocystis carinii pneumonia.

studies were normal; however, he had previously had creatinine level of 3.1 on 27 May 2000 for less than 48 h. On 24 June 2000 he experienced progressive difficulty in breathing. On 27 June 2000, the patient was dyspneic (36 breaths/minute) despite good oxygen saturation, clear lungs and a normal chest X-ray.

On 3 July 2000, the dyspnea persisted without cough, fever, or chest pain. He had oral thrush, a tender liver and chronic leg edema. Platelets were 23 000/μl without schistocytes and he had lactic acidosis. He developed serosanguinous bilateral pleural effusions with negative cultures and possible ascites. He was treated empirically for *Pneumocystis carinii* pneumonia with clindamycin, primaquine, and methylprednisolone. The antiretroviral medications and trimethoprim/sulfamethoxazole were maintained. The patient received one unit of platelets, furosemide, and bumetanide. Repeat laboratory findings were: absolute neutrophil count 1.1, platelets 23 000/μl, sodium 120 mEq/l, bicarbonate 13 mEq/l and creatinine 3.9 mg/dl. He was persistently febrile with negative cultures. The patient died on 7 July 2000.

We have described two patients who received SU5416, a potent inhibitor of VEGF-mediated Flk-1 receptor signaling, who developed unexpected thrombocytopenia, renal failure and death. The exact pathophysiology is unknown. Autopsies were not performed. The advanced AIDS, multiple antiretroviral medications, chronic anemia, and, in the second patient, a previously resolved and rather brief renal insufficiency, are many variables that may have interacted.

In the first patient a precipitous increase in HIV viral load accompanied the hepatorenal failure and thrombocytopenia. In the second patient, abacavir and stavudine have rare renal toxicity and may have contributed to acute renal failure and acidosis. Multi-organ thrombosis including pulmonary embolism may have been the cause of death given the dyspnea, low-grade fever, and leg edema. Nonetheless, in neither patient were schistocytes noted, a requisite for a diagnosis of thrombotic thrombocytopenic purpura. Progressive renal failure played a role in both cases. The bilateral pleural effusion and ascites in patient 2 suggested a capillary leak syndrome. SU5416 administration has also been associated with a risk of thrombosis when given in combination with paclitaxel [2].

Before this study, experience with SU5416 in the setting of HIV infection was limited. Only one of 69 patients enrolled in other SU5416 phase I protocols had AIDS, but that patient did not have significant toxicities. Moreover, although thrombosis was reported in less than 10% of patients in phase II trials of SU5416, no cases of renal failure or death were reported in 200 patients [3–6]. An expanded safety SU5416 database ($n = 393$) revealed five previous cases of grade III/IV transient renal failure or acidosis, all related to volume depletion or gentamicin. However, unique toxicities may occur in the setting of HIV, contributing to infection and mortality, as seen in a subset of patients with low CD4 cell counts receiving combined rituximab and cyclophosphamide–hydroxydaunorubicin–vincristine–prednisone chemotherapy [7]. Similarly, patients with AIDS-associated KS treated with imatinib had unanticipated diarrhea at doses usually tolerated in other cancer patients [8]. Finally, taxanes were associated with increased toxicity in the setting of hepatic cytochrome CYP3A4 inhibition by the concomitant administration of azole antifungal agents [9] or delavirdine and saquinavir [10]. Taken together, these observations suggest that patients with HIV infection should be evaluated separately for toxicity on clinical trials of investigational agents.

Ariela Noy[a], Jamie von Roenn[b], Alex Politsmakher[c], Steven Miles[d] and Claus Bernd[e], *[a]Memorial Sloan-Kettering Cancer Center, New York, NY, USA; [b]Northwestern University School of Medicine, Chicago, IL, USA; [c]Methodist Hospital Medical Center, Brooklyn, NY, USA; [d]University of California, Los Angeles, CA, USA; and [e]Klinik A, Ludwigshafen, Germany.*

Sponsorship: This study was partly supported by cooperative agreements from the National Institutes of Health and National Cancer Institute (U01CA70062 U01CA71375, U01CA70019, U01CA70054, U01CA70080).

Received: 17 March 2006; revised: 14 July 2006; accepted: 8 August 2006.

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A delayed immune reconstitution inflammatory syndrome

The immune reconstitution inflammatory syndrome (IRIS) among HIV-infected patients initiating combination antiretroviral therapy (CART) and pretreated for an opportunistic infection is commonly described [1–3]. In the case of HIV patients receiving both antituberculous and antiretroviral drugs, it seems to be quite a frequent event, concerning 29–36% of patients [4]. In multivariate analysis, the independant reported risk factors for IRIS are disseminated tuberculosis, the timing of the initiation of CART after tuberculosis treatment, a rapid increase in CD4 cells or decrease in the viral load greater than 2 log copies/ml under CART within a month [1,5,6]. The currently admitted physiopathological basis of IRIS is a sudden CART-mediated increase in antigen-specific cells, which in turn induces an explosive response against mycobacterial antigen [3,7]. Usually IRIS occurs when the delay between antituberculous therapy and CART initiation is approximately 2 months, with a maximal reported latency of 40 weeks [8].

We describe here a case of possible IRIS occurring more than a year and a half after the initiation of antituberculous treatment.

A 43-year-old Laotian man, seropositive for HIV-1 but not yet treated, presented with pulmonary tuberculosis with a large cavity in the right upper lobe in April 2004, and soon after began his treatment with a combination including rifampicin, isoniazid, ethambutol and pyrazinamid. The absolute number of CD4 cells at the initiation of antituberculous treatment was 261 cells/μl. The patient remained contagious for 6 months, as a result of inadequate drug concentrations. The dosage was progressively increased, adapted to blood drug concentrations, until a dosage corresponding to a weight of 60 kg was reached, although the patient weighed only 35 kg. Three other antituberculous drugs were added to avoid the selection of a resistant strain during the period of inadequate drug concentration. The last positive culture of a respiratory specimen was taken in October 2005, and the strain was still responsive to all drugs.

In August, a pneumothorax was drained in the chest surgery department. In October, a fistula appeared on the scar of the drain. This event was considered as the first IRIS, even though the patient was not taking CART, and was treated successfully with steroids (1 mg/kg) for 2 months. The antituberculous regimen was simplified to rifampicin and isoniazid in December, after clinical improvement and 2 months of negative respiratory specimen cultures.

In September 2005, 20 months after antituberculous therapy initiation, CART was introduced, using an combination of nucleoside inhibitors and efavirenz. Just before treatment initiation, the absolute CD4 cell count was 317 cells/μl, and the viral load was greater than 5.7 $\log_{10}$ copies/ml.

Three weeks after the initiation of CART, at the end of October, the patient noticed a fistula on the scar of the chest drain. The absolute CD4 cell count was 422 cells/μl and the viral load was 2.7 $\log_{10}$ copies/ml. Drug concentrations (rifampicin and efavirenz) were within the therapeutic ranges. All the microbiological specimens (from the fistula and respiratory tract) were negative for tuberculosis. A computed tomography scan did not show any evidence of tuberculosis reactivation. The fistula disappeared spontaneously in one month, at the end of November.

The patient was hospitalized in the same period to explore asthenia, weight loss and a cough lasting for a month. Semi-invasive aspergillosis was diagnosed in the sequellar cavity on the basis of microbiological and radiological findings (see Fig. 1). The specific treatment was introduced on 9 December and symptoms progressively disappeared.

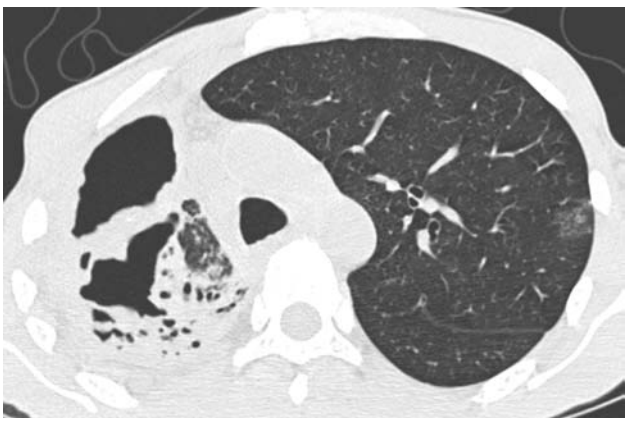

Fig. 1. Computed tomography scan showing area of consolidation with a new cavitation in the right upper lobe.

The fistula could be considered as a 'possible' IRIS under the generally admitted definition [4,6]. It was a reappearance of a previous tuberculosis manifestation (fistula), despite effective antituberculous therapy (correct rifampicin concentration), after the exclusion of a relapse of tuberculosis or other diagnosis (negative microbiological examination for both tuberculosis or other microorganisms). It occurred 3 weeks after the initiation of CART, which delay is similar to those usually reported [4,6,8]. It also occurred in the context of rapid immune-virological improvement, 3 weeks after the initiation of CART. A histological examination of the products of the fistula was not performed, but could have provided more evidence of IRIS by showing inflammation mediated by T cells.

This report is unusual in the unusually long delay between the antituberculous treatment and the initiation of CART. In the literature, we did not find any report of such latency between the initiation of antituberculous treatment and the occurrence of IRIS.

In our case, we could hypothesize that the patient had a weak specific immune response against tuberculosis, perhaps constitutional, and was not able to clear the tuberculous antigens rapidly. He may have also presented with a primary disseminated tuberculosis. Therefore, it is possible that tuberculosis antigens remained numerous even after 20 months of treatment, especially in the context of an insufficient dosage of antituberculous drugs during the initial months, leading to an IRIS when CART was initiated [3].

We also have to bear in mind the diagnosis of IRIS even after a long time after antituberculous treatment initiation, to propose therapy based on steroids in the case of severe symptomatology.

The diagnosis of aspergillosis could also have been considered as IRIS in this context, as suggested by others [9], but with the lack of evidence of latent aspergillosis before CART initiation, it was not possible to validate this hypothesis. This hypothesis seems interesting, but more observations need to be gathered. Fungal examinations should be systematically proposed in these patients with significant sequellar cavities of tuberculosis, mainly before CART initiation.

Johann Cailhol[a,c], Cecilia Pizzocolo[a,c], Michel Brauner[b], Olivier Bouchaud[a,c] and Sophie Abgrall[a,c,d],
Assistance Publique Hopitaux de Paris, Departments of [a]Infectious and Tropical Diseases, [b]Radiology, Avicenne Hospital, Bobigny, F-93000 France, [c]Université Paris 13, Paris Nord, Bobigny F-93017, France, and [d]INSERM U720, Université Pierre et Marie Curie, Paris 6, Paris F-75013, France.

Received: 26 May 2006; accepted: 14 September 2006.

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Basal RANTES promoter activity differs considerably among different species of monkeys: implications for HIV-1/AIDS progression

Regulated upon activation: normal T-cell expressed/secreted (RANTES) is an important chemokine that interacts with the chemokine receptor CCR5 (HIV-1 co-receptor). It is important to study the genetic differences in the promoter region of the RANTES gene in different species of monkeys because it is used as an animal model to undestand HIV-1/SIV pathogenesis and also for vaccine studies. We present varying levels of basal promoter activity that may impact the progression of HIV/SIV.

The chemokine system plays an important role in modulating viral entry, tropism and pathogenesis [1–3]. Earlier studies had clearly established that β-chemokines (RANTES, macrophage inflammatory proteins, ligands for chemokine receptor CCR5) as well as others could

interfere with the interaction of host cells with HIV-1 [3]. The protective effects of IL-4 -589T and RANTES -28G on HIV-1 disease progression have recently been reported [4]. SIV also exploits the chemokine system to infect certain species of monkeys [2]. Besides, monkeys are used as an animal model for understanding the pathogenesis of SIV/HIV and also for AIDS vaccine-related studies. Based on the species of monkeys used for HIV/SIV challenge, they produce varying pattern of disease, and the genetic basis for these differences needs to be studied. With this aim, we sought to carry out a comparative study on the basal promoter activity of the RANTES promoter of several species of monkeys simultaneously on human and monkey cells. Earlier studies in humans identified two mutations (−28G and −403A) in the *cis*-acting regions (promoter) of RANTES that controlled the progression of HIV-1 [5]. We amplified the promoter region of RANTES according to the method described earlier by us [6]. In that communication we reported the presence of four highly polymorphic regions (A–D) in the promoter region (spanning +78 to −413) of several monkeys. We hypothesized that such changes could potentially modulate the production of RANTES. The following primer pairs with *Xho*1 and *Hind*III restriction sites, respectively, were engineered in the beginning of forward and reverse primers for carrying out polymerase chain reaction (PCR):

Forward 5′–GGC–CTCGAG–GGACCCTCCTCAA TAAA–3′
Reverse 5′–GGC–AAGCTT–GCGCAGAGGGCAG TAGCAAT–3′

The sequences underlined represent *Xho*1 and *Hind*III restriction sites. PCR amplified products were cloned into the PGL3 luciferase reporter vector (Promega Biotech, Wisconsin, USA). This vector is designed to provide information about the intracellular activity of the promoter. The PCR product (604 bases) was isolated from the gel using the QIA-quick gel extraction kit (Qiagen, Hilden, Germany), digested with *Xho*1 and *Hind*III and ligated into the PGL3 vector digested with the same two restriction enzymes using T4 DNA ligase (Promega). Equal amounts of Qiagen column purified plasmid DNA (0.4 μg) per 0.1×10^6 cells in a 12-well plate were transfected along with an internal control (pSV-β-gal; Promega), which uniformly gave 70–80% transfection efficiency. Both human (Magi-CCR5, obtained from the National Institutes of Health AIDS reagent programme) and monkey cells (Cos-1) were transfected for 24 h with lipofectin (Gibco BRL, Eggenstein, Germany), and cell lysates were prepared for the detection of luciferase activity using a kit from Promega (luciferase assay system). Relative luciferase activity obtained with two normal humans and four species of monkeys are shown in Fig. 1(a and b). They are mean ± SD from four different experiments. Luciferase units were normalized and compared against the values obtained by transfecting only the vector PGL3. The pattern of promoter activity was essentially the same for the two cell lines used. Both normal humans showed very little basal activity in Cos-1 and human cells. Langur monkeys (*Prebytes entellus*) showed approximately twofold more activity in Cos-1 compared with its activity in human cells. Remarkably, both rhesus (*Macaca mulatta*) and marmoset (*Callith rix jacchus*) monkeys always showed significantly more promoter activity (approximately fivefold in Cos-1 and threefold in human cells). In contrast, bonnet monkeys showed significantly less basal promoter activity in both cell lines.

We report for the first time variations in the basal RANTES promoter activity in four commonly used species of monkeys, and show that they differ quite significantly from each other. These observations are important because RANTES levels have earlier been

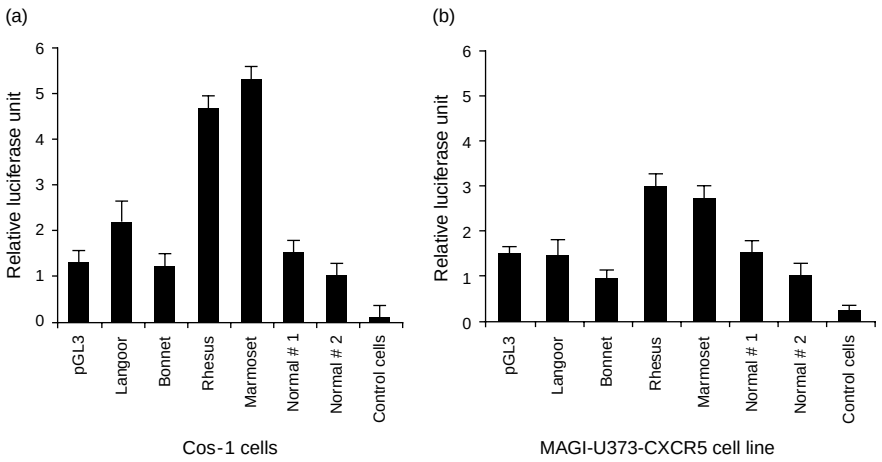

Fig. 1. An approximately 604 bp fragment containing the essential RANTES promoter elements was amplified as described earlier. They were cloned into the PGL3 enhancer containing vector. They were transfected into human and mouse cells as described in the text and luciferase activity was determined from equivalent amounts of lysates using a kit from Promega.

shown to modulate the progression of HIV-1. As it is one of the major chemokines for the CCR5 receptor, it may also be involved in other immune-mediated disorders/diseases.

Received: 15 July 2006; accepted: 26 September 2006.

Acknowledgement

The authors would like to thank Subeer Majumdar for providing blood samples from the Primate Research Center of the National Institute of Immunology, New Delhi, India.

Vikas Sood, Nidhi Gupta, Vijay Shanker, Aalia S. Bano and Akhil C. Banerjea, *National Institute of Immunology, JNU Campus, New Delhi 110067, India.*

Sponsorship: Grants received by the Department of Biotechnology, to the National Institute of Immunology, New Delhi, and to A.C.B. are gratefully acknowledged.

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Caspofungin-resistant oral and esophageal candidiasis in a patient with AIDS

Oropharyngeal candidiasis caused by *Candida albicans* is one of the most common opportunistic infections in patients with advanced HIV infection [1,2]. Azoles, such as fluconazole, are commonly used to treat these infections; however, resistance of *C. albicans* to azoles is problematical [2–4]. Treatment options for azole-resistant oral and esophageal candidiasis remain limited. Caspofungin, an echinocandin with activity against *Candida* species targeting the synthesis of beta-1, 3-glucan in the fungal cell wall [5], expands the treatment options for azole-resistant oral–esophageal candidiasis in patients with AIDS [4,5]. Resistance to caspofungin remains uncommon. A patient with AIDS and azole-resistant oral–esophageal candidiasis caused by *C. albicans* is described who developed clinically significant resistance to caspofungin despite conflicting minimum inhibitory concentration (MIC) results.

A 44-year-old man with advanced HIV/AIDS infection presented with azole-resistant oral–esophageal candidiasis caused by *C. albicans*. The patient had previously been treated sequentially with oral nystatin solution, fluconazole, and itraconazole solution without a favorable response. The patient's CD4 cell count had been less than 10 cells/μl for more than 4 years, and the viral load was greater than 100 000 copies/ml. HIV genotyping and phenotyping showed significant resistance to all three main classes of antiretroviral agents. Additional co-morbidities included hepatitis B infection, perianal human papillomavirus infection and squamous cell carcinoma, hypertension, and renal insufficiency (estimated creatinine clearance 75 ml/min).

Caspofungin treatment was begun with a loading dose of 70 mg intravenously once, followed by 50 mg intravenously a day for 14 days. The patient tolerated the medication well and examination showed marked clearing of the oral lesions. His appetite improved and he gained weight. Caspofungin treatment was re-initiated 3 months later as a result of a recurrence of symptoms. After his second course, he again showed improvement, which persisted a few months. He subsequently received five additional 2-week treatment courses, administered over one year. These treatment courses were periodically interrupted and prematurely terminated as a result of missed infusion appointments. The clinical effectiveness of the medication gradually diminished. After the seventh and final course of caspofungin, the plaques showed no change and the patient experienced no clinical improvement. Shortly thereafter the patient moved to a new geographical area.

Only *C. albicans* was cultured from oral swabs taken periodically throughout the patient's treatment course; all isolates showed high-level resistance to fluconazole. The MIC of caspofungin calculated from the first sample obtained before the initiation of therapy was 0.13 μg/ml or less, comparable to that seen in fluconazole-resistant and caspofungin-susceptible *C. albicans* [6]. Susceptibility testing showed an MIC of 0.03 μg/ml after the fourth course of caspofungin (approximately 11 months after the first treatment), at which time resistance was clinically suspected, and an MIC of 1.0 μg/ml during the sixth treatment course, approximately 16 months after

beginning treatment. At both points in time the patient showed increasing numbers of persistent oral plaques despite caspofungin treatment, in contrast to his response during his initial course. After his seventh and final course of caspofungin, approximately 19 months after his first course, the MIC was less than 0.03 μg/ml.

This patient with AIDS and azole-resistant oral–esophageal candidiasis demonstrated clinically progressive resistance to caspofungin, despite conflicting susceptibility results. It is possible, and likely, that this patient's treatment interruptions, incomplete treatment courses, and the prolonged duration of treatment hastened the onset of treatment failure and clinical resistance. Before treatment, the caspofungin MIC was low as expected, and was consistent with that found in *C. albicans* strains known to be caspofungin susceptible. Testing performed later continued to show an unexpectedly low MIC, despite the fact that the patient showed signs suggestive of treatment failure. Caspofungin-resistant *Candida* species typically have an MIC at least greater than 1.0 μg/ml (L.C. Sadowski, personal communication). Hernandez *et al.* [7] described a caspofungin MIC greater than 64 μg/ml in the setting of caspofungin treatment failure in an AIDS patient with *C. albicans* esophagitis. Kartonis *et al.* [8] also reported no correlation between caspofungin MIC levels and clinical outcomes in patients with esophageal candidiasis or invasive candidiasis. Although it is possible that pharmacokinetic variability was a factor in this patient, the patient's initial positive response, the standardized dosing, and the length of time between dose administrations suggest that this would be unlikely.

This report highlights the importance of recognizing the possibilities of clinical resistance of *C. albicans* to caspofungin and the lack of a close relationship between MIC levels and clinical resistance. Genetic and enzymatic analysis of *C. albicans* isolates in patients failing caspofungin treatment could further the understanding of mechanisms of resistance and susceptibility testing methodologies, and provide new directions for the development of novel therapies.

Janet A. Jokela and Preeti Kaur, *VA Illiana Health Care System, Danville, Illinois, USA; and University of Illinois at Urbana–Champaign College of Medicine, Urbana, Illinois, USA.*

Received: 17 July 2006; accepted: 3 October 2006.

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Nifedipine–lopinavir/ritonavir severe interaction: a case report

Lopinavir is administered co-formulated with a low ritonavir dose (a potent cytochrome P450(CYP)3A4 inhibitor). As lopinavir is predominantly metabolized via cytochrome P450(CYP)34A, the subtherapeutic dose of ritonavir inhibits lopinavir metabolism resulting in higher lopinavir concentrations, especially higher trough levels [1,2].

Nifedipine (a dihydropyridine calcium antagonist) is also a cytochrome P450(CYP)34A substrate [3]. The co-administration of ritonavir with nifedipine may significantly increase serum concentrations of nifedipine resulting in toxicity: headache, peripheral oedema, hypotension and tachycardia. Although this interaction has only a theoretical substantiation, it is well described as a low grade, usually clinically irrelevant interaction [3]. We report a case of a nifedipine–low-dose ritonavir interaction with severe hypotension and renal failure confirmed with an unintended re-exposition to the drugs.

A 47-year-old man was diagnosed with HIV infection in 1992. His medical history included two bouts of *Pneumocystis jiroveci* pneumonia, chronic hepatitis C and

hypertension treated with nifedipine 30 mg/12 h, doxazosine 1 mg/12 h and furosemide 40 mg/8 h. HAART (estavudine 40 mg/12 h, lamivudine 300 mg/24 h and lopinavir/ritonavir three pills/12 h) was begun in 2002. In March 2005, hypotension and progressive renal failure were noted; HAART was withdrawn, when the CD4 cell count was 105 cells/μl, and a kidney biopsy confirmed membranoproliferative glomerulonephritis.

In May 2005, during his follow-up, lopinavir/ritonavir re-introduction was decided when he was receiving nifedipine 30 mg/12 h, doxazosine 1 mg/12 h and furosemide 40 mg/8 h.On the second day the patient developed malaise, severe hypotension (90/50 mmHg), oliguria, and progressive generalized oedemas. Serum creatinine was 5 mg/dl (creatinine before HAART re-introduction was 1.7 mg/dl). All antihypertensive and antiretroviral drugs were discontinued, and the patient was managed with symptomatic treatment and hydroelectrolitic repletion. Renal function progressively improved, the creatinine level lowered to 3 mg/dl, the clinical symptoms vanished and blood pressure normalized. The episode was diagnosed as prerenal acute kidney failure and antihypertensive drugs were re-introduced one week later, first nifedipine 30 mg/12 h, and later enalapril 5 mg/12 h and losartan 50 mg/12 h. At that time the patient was clinically stable, with good control of blood pressure and a serum creatinine level of 1.7 mg/dl. One week later the re-introduction of lopinavir/ritonavir was decided. Again, after 48 h another hypotension episode occurred, with malaise, oedemas and renal insufficiency (creatinine peak 7 mg/dl). Every drug was stopped and in approximately 72 h a progressive clinical and biochemical improvement was noted.

As an interaction between HAART and antihypertensive drugs was suspected, in June 2005 only HAART was re-introduced. The patient went on improving, reaching a creatinine level of 2.5 mg/dl. Amlodipine 15 mg/24 h, a dihydropiridine calcium antagonist metabolized via CYP314, was substitued for nifedipine. Two week later the patient was discharged with creatinine 2.8 mg/dl and a diagnosis of severe hypotension with secondary renal failure caused by a pharmacologic interaction between nifedipine and HAART. During a 6-month follow-up the patient was clinically stable with well-controlled hypertension and a creatinine level of 2 mg/dl.

We describe a case of acute renal insufficiency with severe hypotension and oedema caused by a pharmacokinetic interaction. When nifedipine is administered to a patient receiving ritonavir, CYP450 is inhibited and this can lead to a high nifedipine trough level, resulting in hypotension and renal failure, especially in patients with some degree of renal insufficiency. Hypotension can induce a quick and severe renal failure in patients with mild renal insufficiency. As we could not find a suitable cause for the first hypotension episode, HAART and antihypertensive drugs were re-introduced after clinical improvement. This fuelled another hypotension episode that confirmed the pharmacokinetic interaction between ritonavir and nifedipine through an unintended re-exposition. At this point we decided to associate stavudine, lamivudine and lopinavir/ritonavir with amlodipine, a drug with a metabolism not affected by ritonavir [3] and it was well tolerated.

Pharmacokinetic ritonavir interactions can result in clinically meaningful problems, at least with ketoconazole, itraconazole, amiodarone, quinidine, atovaquone, nicardipine and methadone. The levels of all those drugs, and their effects, can be greatly heightened by ritonavir. As has been shown, a nifedipine–ritonavir interaction can lead to serious clinical problems. At this time it is not known whether there is any nifedipine dose that can be used safely when co-administered with ritonavir. As lopinavir is available only co-formulated with ritonavir, it is difficult to ascertain the differential CYP inhibitory effects of lopinavir (if any) and ritonavir in this interaction. Further research, especially in patients receiving many drugs and with co-morbidities, is needed if ritonavir and nifedipine are going to be used together in the future [4].

To the best of our knowledge, this is the first description of a severe interaction between nifedipine and ritonavir leading to prerenal kidney failure induced through severe hypotension.

María T. Baeza[a], Esperanza Merino[a], Vicente Boix[a] and Eduardo Climent[b], *[a]Infectious Diseases Unit, and [b]Pharmacokinetics Department, Hospital General Universitario de Alicante, Pintor Baeza, 12, 03010 Alicante, Spain.*

Received: 7 July 2006; revised: 18 July 2006; accepted: 3 October 2006.

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National adult antiretroviral therapy guidelines in South Africa: concordance with 2003 WHO guidelines?

We read with interest the article by Beck and colleagues [1] who examined the adult antiretroviral therapy (ART) guidelines in 43 World Health Organization (WHO) '3 by 5' focus countries. The authors found that the national guidelines of a majority of countries had a good degree of concordance with the WHO 2003 guidelines [2]. Although concordance was noted to be inversely related to health expenditure per capita, the authors did not further explore the reasons why some countries have adopted guidelines that differ from the current WHO recommendations. One such country is South Africa, which has among the highest per capita income of countries in subSaharan Africa and also has much better healthcare infrastructure than most. Despite these resources, the South African national ART programme currently bases its treatment guidelines on the former WHO 2002 guidelines that recommend ART only for patients with WHO stage 4 disease (AIDS) or a blood CD4 cell count of less than 200 cells/μl [3]. We believe these guidelines advocate treatment at too late a stage of disease and that they represent a compromise that may substantially undermine the effectiveness of the programme in the long term.

The key goals of an ART programme are to prevent morbidity and mortality. We have recently demonstrated in a historical natural history cohort in Cape Town, South Africa, that 52% of HIV-associated deaths actually occur before the development of stage 4 disease [4]. Moreover, the rate of progression to AIDS among patients with CD4 cell counts of 200–350 cells/μl was almost twice that reported in natural history cohorts in high-income countries [4,5]. Therefore, under the current South African national guidelines, much HIV-associated morbidity and mortality in this setting occurs among patients before the eligibility for ART.

Data from ART programmes also suggest that treatment is being initiated too late. Within a community-based programme in Cape Town, the mortality rate among referred individuals eligible to start ART is extremely high, exceeding 30 deaths per 100 person-years [6]. Moreover, during 3 years follow-up of this cohort, 87% of mortality occurred in the interval just before treatment initiation or during the first 16 weeks of ART [7]. Collectively, these data indicate that patients are arriving with disease that is too far advanced. More worryingly, these data are likely to be indicative of a huge burden of mortality occurring upstream of the ART programme.

South Africa is at the epicentre of the continent's HIV-associated tuberculosis epidemic, with tuberculosis notification rates in many townships reaching almost unprecedented levels [8]. The extent to which ART may assist in tuberculosis control at the community level is highly dependent upon how early ART is initiated and upon the extent to which ART reduces the incidence of tuberculosis during long-term treatment [9]. Under the South African ART guidelines, pulmonary tuberculosis (WHO stage 3 disease) *per se* is not a criterion for the consideration of ART. This may well represent a critical missed opportunity; by the time our patients initiate ART, 52% of them have already completed treatment for one or more episodes of tuberculosis and 25% are receiving treatment for current tuberculosis [10]. The later the initiation of ART, the greater the burden of tuberculosis that accrues. Moreover, the long-term incidence of tuberculosis during ART in this cohort remains five to 10-fold higher than that among HIV-non-infected individuals despite excellent virological outcomes [10]. This is likely to reflect the suboptimal restoration of tuberculosis-specific immunity, which is a further factor strongly associated with the late initiation of ART [11]. In view of these outcomes, the late initiation of ART could paradoxically undermine tuberculosis control in South Africa [12].

Clearly, in addition to medical factors, economic and logistic constraints also affect the ART eligibility criteria set by different national ART programmes. However, economic data also indicate the benefits of earlier treatment. Cost-effectiveness analyses using local data from Cape Town indicate that it is more cost effective to treat patients with less advanced disease, although the overall investment is greater [13,14]. Collectively, these data point in the same direction, favouring earlier initiation of ART than is at present recommended in South Africa. Good medical practice mandates that treatment guidelines should not be 'cast in stone' but should be continually reviewed and revised in the light of emerging data. We conclude that the six million people living with HIV in South Africa might be much better served by the national ART guidelines being brought up to date with current revised WHO guidelines. As Beck and colleagues [1] point out, the WHO guidelines have been developed so that 'an effective and appropriate standard of care can be maintained'.

Stephen D. Lawn[a,b] and Robin Wood[a], *[a]The Desmond Tutu HIV Centre, Institute for Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa; and [b]Clinical Research Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK.*

Sponsorship: S.D.L. is funded by the Wellcome Trust, London, UK, and R.W. is funded partly by the National Institutes of Health, USA, RO1 grant A1058736-01A1.

Received: 25 July 2006; accepted: 13 September 2006.

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