Octamer Transcription Factors Up-regulate the Expression of CCR5, a Coreceptor for HIV-1 Entry*

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T cell activation can induce expression of CCR5, a major coreceptor for macrophage-tropic (R5) human immunodeficiency virus type 1 (HIV-1). Here we report that overexpression of the Oct-2 transcription factor and octamer coactivator BOB.1/OFB/OCA-B, both of which are induced in T cells following T cell receptor signaling, synergistically up-regulates CCR5 promoter activity via interaction with an octamer motif on the promoter. We also show that the octamer transcription factors can increase cell surface expression of CCR5 and fusogenicity of the cells with R5 HIV-1 Env. These results suggest that octamer transcription factors may play a critical role in the induction of CCR5 expression on, and thereby susceptibility to, R5 HIV-1 of T cells following antigenic stimulation.

Oct-2 is a transcription factor that binds specifically to octamer motifs on its target promoter and/or enhancer regions including immunoglobulin and interleukin (IL)1-2 genes (1). Oct-2 is expressed predominantly in the B-cell lineage; however, recent studies demonstrated that B cell-restricted gene regulation is dependent on B cell-specific coactivator BOB.1/OFB/OCA-B (2–5). Expression of Oct-2 and BOB.1/OFB/OCA-B is also induced by antigenic stimulation of T cells (6, 7). Thus, the octamer transcription system may play a role in gene expression during T cell activation.

The CC chemokine receptor CCR5 is preferentially expressed in type 1 T helper cells (8) and serves as a receptor for the CC chemokines regulated upon activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein 1α and 1β (9–11). CCR5 is also essential for cellular entry of macrophage-tropic (R5) human immunodeficiency virus type 1 (HIV-1) (12–17), and levels of its expression appear to be critical for infectability by R5 HIV-1 (18). We have recently cloned and characterized the promoter region of CCR5 and identified an octamer motif on the promoter region (19); however, it remained to be determined whether the octamer motif has a role in the regulation of CCR5 expression on T cells or macrophages, the two major target cells for HIV-1.

In this study, we demonstrate that the octamer motif plays a critical role in the induction of CCR5 expression in activated T cells and that octamer-mediated overexpression of CCR5 increases susceptibility of the cells to R5 HIV-1 infection.

EXPERIMENTAL PROCEDURES

Plasmids—Plasmid pGL-CCR5 WT contains the CCR5 promoter sequence spanning −770 to +61 relative to the transcription start site (19), followed by the luciferase reporter gene. Plasmid pGL-CCR5 3Oct has mutations (shown in boldface letters below) on the octamer motif (indicated by underline) around −230 relative to the transcription start site (ATGAATTTAATGTTTC→ATGAATTTATGTTTC), which was generated by PCR-oriented site-directed mutagenesis (20). Plasmids pCG-Oct2, pCGN-OCA-B, and their parent plasmid pCGN were generous gifts of W. Herr (Cold Spring Harbor Laboratory) (21, 22). Plasmid pMT-GATA1 and its parent plasmid pMT2T were kindly provided by S. H. Orkin (Harvard Medical School) (23); plasmid pMT-p65 (which encodes the NF-κB p65 subunit) was a gift of U. Siebenlist (NIAID, National Institutes of Health) (24). Plasmids encoding Env from HIV-1 R5 strain JR-FL or X4 strain HXB2 or Env from amphotropic murine leukemia virus (AMLV) were kindly provided by N. Landau (Aaron Diamond AIDS Research Center) (14).

Cells—Peripheral blood mononuclear cells were obtained from healthy volunteers (Department of Transfusion Medicine, Warren Grant Magnuson Clinical Center, National Institutes of Health and Red Cross Blood Center in Nagasaki Prefecture, Japan), and CD4+ T cells were isolated as described previously (25). Where indicated, CD4+T cells were stimulated with phorbol 12-myristate 13-acetate (PMA) (1 μM) plus ionomycin (1 μM) or anti-CD3 plus anti-CD28 antibodies immobilized by immunomagnetic beads for 8 h. CCR5-expressing PM1 lymphoid cells were propagated as described previously (19).

RNA-PCR—Total cellular RNA was extracted from cell pellets according to the manufacturer’s instructions (RNasey Mini; Qiagen, Santa Clarita, CA). RNA was reverse transcribed in a 50-μl reaction containing 50 mM Tris-HCl (pH 8.4), 75 mM KCl, 3 mM MgCl2, 10 mM dithiothreitol, 0.8 mM each dNTP, 1 unit of RNasin (Promega, Madison, WI), 100 pmol of random hexamer oligonucleotides, 2 μg of RNA sample, and 400 units of Moloney murine leukemia virus reverse transcriptase (Promega). The reaction was incubated for 60 min at 37 °C, heated at 95 °C for 5 min, and quick-chilled on ice. The cDNA corresponding to whole coding sequence of Oct-2 or BOB.1/OFB/OCA-B was amplified by PCR for 30 cycles in a 50-μl reaction containing 1 μl of cDNA product and 1.25 units of Taq Bead™ Hot Start Polymerase (Promega). MgCl2 concentration in the reaction was 3.0 mM for Oct-2 amplification and 2.0 mM for OCA-B amplification. Primers used for PCR were 5′-ATGCTTAAGCCCTGAGG-3′ (sense) and 5′-CATCAAGGCAGTTAGGC-3′ (antisense) for Oct-2 and 5′-CACACGCTCGGAG-3′ (sense) and 5′-CTAAAAAGCTTCCAGAG-3′ (antisense) for OCA-B. A thermal cycle profile was denaturation for 45 s at 94 °C, annealing for 1 min at 55 °C, and elongation for 1 min 30 s at 72 °C, followed by a final elongation at 72 °C for 7 min. Primers used for amplification of CCR5 cDNA were 5′-TACAGGCTCTGTTACCATTC-3′ (sense) and 5′-TTATATTCTCACCTCAGGC-3′ (antisense). Control amplification was performed for β-actin cDNA. Amplification was performed at denaturation for 45 s at 94 °C, annealing for 1 min at 50 °C, and elongation for 1 min at 72 °C for 20 cycles, followed by a final elongation at 72 °C for 7 min. The PCR products (10 μl for Oct-2, BOB.1/OFB/OCA-B, or CCR5 and 2 μl for β-actin) were resolved on a

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The abbreviations used are: IL, interleukin; RANTES, regulated upon activation, normal T cell expressed and secreted; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; AMLV, amphotropic murine leukemia virus; PMA, phorbol 12-myristate 13-acetate; rVV, recombinant vaccinia virus (es).
1% agarose gel and visualized by ethidium bromide staining.

**Transfection and Transient Expression Assays**—Transfections and luciferase assays for transient expression assays were performed as described previously (26).

**Flow Cytometric Analysis**—Cell surface expression of CCR5 or CXCR4 was determined by staining cells with monoclonal anti-CCR5 antibody 2D7 phycoerythrin conjugate or anti-CXCR4 antibody 12G5 phycoerythrin conjugate (PharMingen, San Diego, CA), respectively, and by analyzing in a FACScan (Becton Dickinson Immunocytometry, San Jose, CA), as described previously (27).

**Viruses and Fusion Assays**—Recombinant vaccinia viruses (rVV) were propagated as described previously (27). rVV expressing Oct-2 (vOct2) was a generous gift of Dr. P. C. van der Vliet (28). Fusion assays were performed as described previously (28). In brief, 293T cells were seeded onto 60-mm diameter tissue culture plates, transfected with 10 μg of an Env expression vector plasmid using calcium phosphate method, infected with vCB21R (a rVV encoding the lacZ gene under the control of T7 promoter) at a multiplicity of infection of 10 on the following day, and served as fusion effectors. Primary CD4+ T cells obtained from healthy volunteers were infected with vTF7–3 (a rVV expressing T7 RNA polymerase) along with vWT or vOct2 at a multiplicity of infection of 10 each and served as fusion targets. After a 10-h culture at 37 °C, both fusion effectors (293T cells) and fusion targets (CD4+ T cells) were mixed and incubated at 37 °C for 6 h in the presence of cytosine arabinoside, and the mixed cell culture lysates were subjected to β-galactosidase assays.

**RESULTS**

**Activation of CD4+ T Cells Induces Expression of Oct-2 and BOB.1/OBF/OCA-B**—Since we have recently identified an octamer motif on the CCR5 promoter, we wanted to investigate whether this motif plays a role in CCR5 expression on CD4+ T cells, a target for HIV-1. First, we examined whether T cell activation can induce expression of Oct-2 (a B cell-specific octamer factor) and BOB.1/OBF/OCA-B (a B cell-specific octamer coactivator). As shown in Fig. 1, mRNA specific for Oct-2 and BOB.1/OBF/OCA-B was amplified by reverse transcriptase-PCR in CD4+ T cells treated with PMA plus ionomycin or anti-CD3 and anti-CD28 (Anti-CD3/CD28). Total RNA prepared from these cells was subjected to reverse transcriptase-PCR to amplify cDNA for CCR5, Oct-2, BOB.1/OBF.1/OCA-B, or β-actin.

**FIG. 1.** Induction of Oct-2 and BOB.1/OBF.1/OCA-B mRNA expression by activation of CD4+ T cells. CD4+ T cells obtained from normal volunteers were either unstimulated (Control) or stimulated with PMA plus ionomycin or anti-CD3 plus anti-CD28 (Anti-CD3/CD28). Total RNA prepared from these cells was subjected to reverse transcriptase-PCR to amplify cDNA for CCR5, Oct-2, BOB.1/OBF.1/OCA-B, or β-actin.

**FIG. 2.** The octamer motif on the CCR5 promoter mediates Oct-2-induced transactivation. A, 20 million PM1 cells were transfected with 20 μg of pGL-CCR5 WT along with the indicated amount of pCG-Oct2. Total input DNA amounts were adjusted equally by adding pCGN (parent plasmid). Luciferase assays in the transfected cell lysates were performed 40 h after transfection. Results are reported as means ± S.E. from four independent experiments. B and C, 20 million PM1 cells were transfected with 20 μg each of the plasmids indicated at the bottom. Results are reported as means ± S.E. from three independent experiments.
Overexpression of Oct-2 Up-regulates the CCR5 Promoter—To investigate whether octamer transcription factors can regulate CCR5 promoter activity, PM1 cells were transfected with pGL-CCR5 WT along with various amounts of an expression vector for Oct-2. As shown in Fig. 2A, overexpression of Oct-2 up-regulated CCR5 promoter activity in a dose-dependent manner. Furthermore, stimulation with PMA plus ionomycin synergistically increased Oct-2-mediated transactivation of CCR5 promoter (data not shown).

To determine whether an octamer motif on the CCR5 promoter is critical for Oct-2-mediated activation of the promoter, the octamer motif was mutated in plasmid pGL-CCR5 WT, and the construct was tested for its reporter activity. Mutation of the octamer motif had minimal effect on the CCR5 promoter activities induced by either GATA1 (Fig. 2C) or NF-κB p65 (data not shown); however, it almost abolished the promoter activity induced by Oct-2 (Fig. 2B), indicating that the octamer motif plays a crucial role in the Oct-2-induced activity of the CCR5 promoter.

Oct-2 and the Octamer Coactivator BOB.1/OBF.1/OCA-B Synergistically Up-regulate the CCR5 Promoter—The octamer coactivator BOB.1/OBF.1/OCA-B plays a critical role in octamer-dependent transcriptional activation in B cells and activated T cells (2–5, 7). To determine whether BOB.1/OBF/OCA-B plays a crucial role in the induction of CCR5 promoter activity, Oct-2 and BOB.1/OBF/OCA-B were expressed in PM1 cells individually or in combination. Either Oct-2 or BOB.1/OBF/OCA-B alone had a minimal effect on the CCR5 promoter activity at the concentration used; however, expression of both factors markedly enhanced the promoter activity (Fig. 3).

Overexpression of Oct-2 Up-regulates Cell Surface Expression of CCR5—As shown above, octamer transcription factors can up-regulate the CCR5 promoter. To demonstrate whether cell surface expression of CCR5 is also up-regulated by octamer transcription factors, we overexpressed Oct-2 by infecting CD4+ T cells with vOct2 (a rVV expressing Oct-2), and determined cell surface CCR5 expression by flow cytometry. CCR5 expression in CD4+ T cells that were infected with vWT (a wild type rVV) was modest (3%) but was significantly induced (30%) by infection of CD4+ T cells with vOct2 (Fig. 4A). Thus, overexpression of Oct-2 appears to induce cell surface expression of CCR5. In contrast, overexpression of Oct-2 did not induce cell surface CXCR4 expression (Fig. 4B).

Overexpression of Oct-2 Increases Fusogenicity of Peripheral Blood Lymphocytes with R5 HIV-1 Envelope—Levels of CCR5 expression appear to correlate well with infectability of CD4+ T cells by R5 HIV-1 (18). We therefore investigated whether up-regulation of CCR5 expression by Oct-2 can increase efficiency of fusion/entry of R5 HIV-1. Primary CD4+ T cells (fusion targets) were infected with vWT or vOct2 as well as vTF7–3 (expressing T7 RNA polymerase). 293T cells (fusion effectors) were transfected with a plasmid expressing HIV-1 JR-FL (R5) Env or HIV-1 HXB2 (X4) Env or a plasmid expressing AMLV Env and infected with vCB21R (encoding the lacZ gene under the control of the T7 promoter). Fusion efficiency between the two cells was assayed by β-galactosidase activity in the mixed cell culture lysates. Overexpression of Oct-2 markedly increased fusogenicity of CD4+ T cells with R5 HIV-1 Env.

Fig. 3. Oct-2 and the octamer coactivator BOB.1/OBF.1/OCA-B synergistically up-regulate the CCR5 promoter. 20 million PM1 cells were transfected with 20 μg each of pGL-CCR5 WT or pGL-CCR5ΔOct along with 10 μg each of the plasmids indicated at the bottom, and luciferase assays were performed 40 h after transfection. Results are reported as means ± S.E. from four independent experiments.

Fig. 4. Overexpression of Oct-2 increases cell surface expression of CCR5. Primary CD4+ T cells were infected with either vWT (a wild type vaccinia vector) or vOct2 (a vaccinia vector expressing Oct-2) overnight, stained with either anti-CCR5 (A) or anti-CXCR4 (B) phycoerythrin-conjugate (shown in solid lines), and analyzed in FACSscan. Staining with isotype control is shown in broken lines, with shaded areas. Results are representative of three independent experiments.
Fig. 5. Overexpression of Oct-2 increases fusogenic activity of the cells with R5 HIV-1 Env. Primary CD4+ T cells (fusion targets) were infected with vWT or vOct-2 as well as vTF7–3 (expressing the T7 RNA polymerase). 293T cells (fusion effectors) were transfected with a plasmid expressing R5 HIV-1 (JR-FL) Env, X4 HIV-1 (HXB2) Env, or AMLV Env and infected with vCB212 (encoding the lacZ gene under the control of the T7 promoter). The two sets of cells were mixed in the presence of cytosine arabinoside, and the control of the T7 promoter). The two sets of cells were mixed in the presence of cytosine arabinoside, and

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