

CCR5 Promoter Alleles and Specific DNA Binding Factors

At least 12 single nucleotide polymorphisms (SNPs) within the 5' upstream regulatory region of the human *CCR5* chemokine and HIV-1 receptor gene have been described (1-4). Our recent report (1) and others (2) have shown that a common 10-SNP allele haplotype, *CCR5P1*, with demonstrated promoter activity (5) confers relatively rapid progression to various AIDS end points in a genetic epidemiologic analysis of 2603 patients. Although quantitative differences in expression between *CCR5* coding alleles (*CCR5*+ versus *CCR5*-Δ32) are apparent (6, 7), we observed no appreciable constitutive differences among *CCR5* promoter alleles (*P1* and *P4*) in HIV-1 binding, in chemokine-mediated signal transduction, or in *CCR5* quantification (1).

We have found a distinction in specific binding affinity for separate *CCR5P* allele sequence motifs to nuclear binding (potential transcription) factors, which suggests a possible mechanism for *CCR5P1/P1* epidemiologic consequences. We used electrophoretic mobility-shift analysis (8) (EMSA) to assess DNA-protein interactions with the common *CCR5P1* (frequency, *f* = 0.56) and *CCR5P4* (*f* = 0.35) alleles. Synthetic allele-specific oligonucleotides (~20 bp), representing polymorphic *CCR5P* sites 208 (G/T), 627 (C/T), 676 (A/G), and 303 (A/G) (2) in the context of their adjacent nucleotides, were incubated with nuclear extracts from phytohemagglutinin (PHA)-blasted phorbolmyristate acetate (PMA) and ionomycin-treated human T cells (8). Variants at three sites (303, 627, and 676) showed no difference in binding for alternative allele oligonucleotides; however, the T-bearing oligonucleotide at site 208 (carried in *CCR5P3* and *P4*) (1) displayed 5- to 12-fold greater binding to a specific nuclear binding protein (or proteins) than did the G-bearing oligonucleotide (Fig. 1, complex A). Specificity for the binding was demonstrated by the fact that competitive binding of the cold *CCR5P* oligonucleotide (208T and 208G), but not of a nonspecific SP-1 oligonucleotide, eliminated complex A formation. Additionally, in cross-competition experiments with 208G versus 208T, as little as 10-fold excess of cold 208T eliminated complex A formation on 208G. However, 100-fold excess of cold 208G only partially competed for complex A formation on 208T.

We examined the sequence surrounding *CCR5P* site 208 for sequence homology to binding sites for previously described transcription factors (9). The sequence revealed significant homology to sites capable of binding to cRel (a member of the Rel/NF-κB family). We

incubated specific antibodies to cRel (10), p50 (11), and p65 (11) with the 208T oligonucleotide. We then performed EMSAs, and each antibody resolved unique complexes in addition to complex A (Fig. 1, complex C). The 208G allele also produced a weak supershift complex with the three antisera, which indicates that this

sequence is also capable of binding cRel, p50, and p65 (12). The intensity of the supershift complex (complex C) relative to complex A is similar whether 208G or 208T is tested, and neither allele appreciably diminishes the major allele specific complex A, as measured by supershift experiments that use the Rel/NF-κB family antisera. Therefore, it seems the predominant interaction of *CCR5P4* (that is, complex A) involves other yet to be identified binding factors in addition to the three implicated transcription factors.

The identification of differential binding

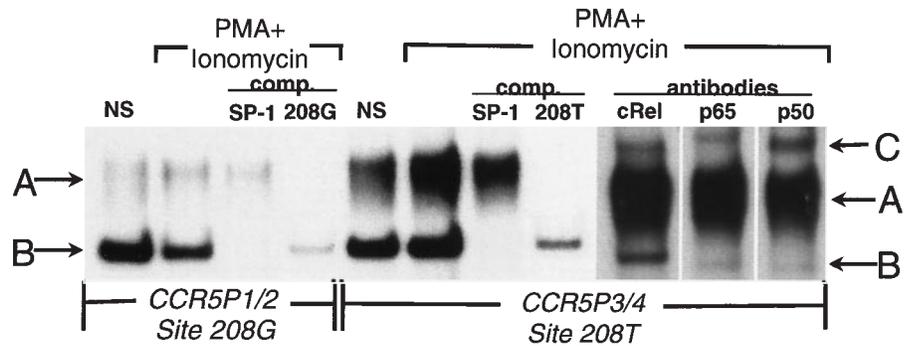


Fig. 1. Differential DNA-protein binding between *CCR5P1/2* site 208G and *CCR5P3/4* site 208T by EMSA with nuclear extracts from PHA-blasted human peripheral blood T cells. Greater DNA-protein binding affinity (complex A) was observed in the 208T site than in the 208G site. NS = non-stimulated; comp. = cold competition with double-stranded oligonucleotides; A = specific complex; B = non-specific complex; C = supershift complex induced by antisera to indicated transcription factors. Complex C specificity was demonstrated by the fact that (i) incubation of radiolabeled oligo and sera alone (no extract) or addition of an irrelevant sera did not resolve the complex, and (ii) peptide competition eliminated complex C formation. Comparable results were obtained with the use of T cell preparations from four distinct human donors. Five gel shift probes were used: 208: AGACAACAGGTTG/TTTTCCGTTTACA; 303: GAGAAAAAGGGGA/GCACAGGGTTA; 627: CGTAAATAAAC/TTCAGACCAG; 676: AGCTCAACTTAAAAA/GGAAGAAGTGTCT; and SP-1: GGGGAGGCGTGGCCTGGGCGGACTGGGGAGTGGCGA.

Table 1. Influence of *CCR5P*-208G variant on survival to AIDS end points (outcomes) when included in genotypes bearing *CCR5P1* or *CCR5P2*. Indicated *CCR5P1* and *CCR5P2* genotypes are on chromosome haplotypes that are wild type (+) for adjacent *CCR2-64I* and *CCR5-Δ32* sites. Results are adjusted for the *CCR5-Δ32* and *CCR2-64I* protective effects as a combined variable in the Cox models.

AIDS outcomes		<i>P1/P1</i>		<i>P1/P2</i> or <i>P2/P2</i>		<i>P1/P1, P1/P2,</i> or <i>P2/P2</i>	
Cohorts	n/events	RH	P-value	RH	P-value	RH	P-value
CD4 < 200							
Caucasians	634/357	1.34	0.06	0.75	0.17	1.07	0.64
MACS	365/192	1.31	0.20	0.84	0.53	1.10	0.59
MHCS	183/119	1.01	0.98	0.57	0.20	0.84	0.48
AIDS 1993							
Caucasians	638/421	1.51	0.005	0.94	0.74	1.25	0.07
MACS	367/242	1.48	0.04	0.94	0.80	1.23	0.20
MHCS	185/126	1.43	0.20	1.00	1.00	1.28	0.32
AIDS 1987							
Caucasians	641/324	1.42	0.03	1.02	0.93	1.26	0.11
MACS	370/191	1.39	0.12	0.97	0.90	1.21	0.29
MHCS	185/89	1.38	0.33	1.44	0.41	1.40	0.24
Death							
Caucasians	641/248	1.31	0.15	1.12	0.64	1.23	0.19
MACS	370/151	1.22	0.40	1.18	0.55	1.21	0.35
MHCS	185/72	1.51	0.24	1.38	0.50	1.47	0.21

TECHNICAL COMMENT

of nuclear factors to oligonucleotides with *CCR5P* site 208T (retained by *CCR5P3* and *P4*) as compared with *CCR5P* site 208G (retained in alleles *CCR5P1* and *P2*) raises the possibility that inclusion of the site 208G in *CCR5P1* (and linkage disequilibrium of the site 303A with *CCR5P1*) would account for the recessive hyper-susceptibility of *CCR5P1/P1* homozygotes to rapid progression to AIDS end points (1, 2). If this were so, then *CCR5P1/P1*, *CCR5P1/P2*, and *CCR5P2/P2* should each be associated with rapid AIDS progression, insofar as both *CCR5P1* and *P2* alleles contain the 208G nucleotide residue (1). We explicitly tested this prediction by comparing cohort survival curves of *CCR5P1/P1* genotypes with *CCR5P1/P2* plus *CCR5P2/P2*, or with the sum of *CCR5P1/P1*, *CCR5P1/P2*, and *CCR5P2/P2* genotypes (Table 1). In every case, the *CCR5P1/P1* genotypes alone were associated with rapid progression, while the *CCR5P2*-bearing genotypes did not progress more rapidly than other *CCR5P* genotypes. For this reason, we conclude that the 208G/T polymorphism differential binding to nuclear factors cannot

fully explain the reported epidemiological data (1, 2).

Nevertheless, the presence of a mixture of nuclear binding factors which discriminate among *CCR5* promoter alleles remains a viable possibility to account for differential availability of *CCR5* receptors in various cell populations. The nuclear factors may vary in abundance among different cell types and respond to diverse stimuli that mediate *CCR5* transcription. Defining the implications of these events is an important goal of ongoing experiments.

Jay H. Bream

Howard A. Young

*Laboratory of Experimental Immunology,
National Cancer Institute—Frederick Cancer
Research and Development Center
(NCI-FCRDC),
Frederick, MD 21702, USA*

Nancy Rice

*Molecular Basis of Carcinogenesis
Laboratory,
Advanced Bioscience Laboratories,
Inc.—Basic Research Program,
NCI-FCRDC*

Maureen P. Martin

Michael W. Smith

Mary Carrington

*Intramural Research Support Program,
Science Applications International
Corporation, NCI-FCRDC*

Stephen J. O'Brien

*Laboratory of Genomic Diversity,
NCI-FCRDC*

E-mail: obrien@ncifcrf.gov

References and Notes

1. M. P. Martin *et al.*, *Science* **282**, 1907 (1998).
2. D. H. McDermott *et al.*, *Lancet* **352**, 866 (1998).
3. S. Mummidi *et al.*, *Nature Med.* **4**, 786 (1998).
4. L. G. Kostrikis *et al.*, *ibid.*, p. 350.
5. S. Mummidi *et al.*, *J. Biol. Chem.* **272**, 30662 (1997).
6. L. Wu *et al.*, *J. Exp. Med.* **185**, 1681 (1997).
7. R. Liu *et al.*, *Cell* **86**, 367 (1996).
8. C. Yu *et al.*, *J. Immunol.* **157** (1996).
9. T. Heinemeyer *et al.*, *Nucleic Acids Res.* **26**, 362 (1998).
10. T-H. Tan *et al.*, *Mol. Cell. Biol.* **12**, 4067 (1992).
11. N. R. Rice *et al.*, *Cell* **71**, 50 (1992).
12. J. H. Bream *et al.*, data not shown.
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Jay H. Bream, Howard A. Young, Nancy Rice, Maureen P. Martin, Michael W. Smith, Mary Carrington and Stephen J. O'Brien

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