Inhibition of HIV-1 Infection by the β-Chemokine MDC

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CD8$^+$ T lymphocytes from individuals infected with human immunodeficiency virus–type 1 (HIV-1) secrete a soluble activity that suppresses infection by HIV-1. A protein associated with this activity was purified from the culture supernatant of an immortalized CD8$^+$ T cell clone and identified as the β-chemokine macrophage-derived chemokine (MDC). MDC suppressed infection of CD8$^+$ cell–depleted peripheral blood mononuclear cells by primary non–syncytium-inducing and syncytium-inducing isolates of HIV-1 and the T cell line–adapted isolate HIV-1_{IIIB}. MDC was expressed in activated, but not resting, peripheral blood mononuclear cells and binds a receptor on activated primary T cells. These observations indicate that β-chemokines are responsible for a major proportion of HIV-1–specific suppressor activity produced by primary T cells.

Activated CD8$^+$ T lymphocytes from HIV-1–infected individuals produce a soluble noncytolytic activity that suppresses infection by HIV-1 (*1*), an effect thought to be specific for this virus. The production of suppressive activity correlates with immune status and decreases gradually in parallel with disease progression (*2*). Major components of this activity responsible for the suppression of macrophage-tropic, non–syncytium-inducing (NSI) isolates of HIV-1 include the β-chemokines RANTES (regulated on activation, normal T expressed and secreted), MIP-1α (macrophage inflammatory protein–1α), and MIP-1β (*3*). However, acute and endogenous infectivity assays performed with either primary T cells (*3, 4*) or macrophages (*5*) as target cells have indicated that the full complement of suppressive activity produced by primary CD8$^+$ T cells is not entirely accounted for by these chemokines. The secretion of RANTES, MIP-1α, and MIP-1β does not correlate with the suppression of certain T-tropic, syncytium-inducing (SI), and T cell line–adapted (TCLA) isolates, and the addition of neutralizing antibodies to these chemokines does not reverse this suppressive effect (*4*). These observations suggest that additional, unidentified chemokines produced by activated T cells are capable of suppressing HIV-1.

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We previously showed that normal CD8$^+$ human T cells immortalized in vitro by human T cell leukemia virus–type I (HTLV-I) are a reproducible source of HIV-1–suppressive activities (*3*). To produce additional such cell lines, but from HIV-1–infected individuals, we transformed CD8$^+$ T cells from seropositive individuals by infection with HTLV-I and cloned the transformed cells by limiting dilution (*6*). The cell clones were then tested for suppressive activity in an acute infectivity assay (*7*) with peripheral blood mononuclear cells (PBMCs) depleted of CD8$^+$ T cells. The assays were performed with primary NSI or SI viruses, or with the TCLA isolate HIV-1_{IIIB}; NSI viruses are sensitive to suppression by RANTES, MIP-1α, and MIP-1β, whereas SI and TCLA viruses are not (*3, 8–10*). The cell clones showed different patterns of suppression when tested with primary NSI or SI isolates or with HIV-1_{IIIB} (Table 1). Two cell clones derived from the same HIV-1–infected individual (F3b clone 3 and F3b clone 19) suppressed the primary NSI isolate (NSI 15) and produced large amounts of β-chemokines. However, only F3b clone 19 suppressed HIV-1_{IIIB} and the primary SI isolate (SI 06). This latter clone was adapted to grow in serum-free medium (*11*) and used for further studies.

The cell-free culture supernatant from F3b clone 19 was subjected to high-speed centrifugation and fractionated by heparin affinity chromatography (*12*), taking advantage of the heparin-binding characteristics of chemokines (*13*). Virtually all of the suppressive activity effective against HIV-1_{IIIB} was recovered in the column eluate (*14*). The eluate was further fractionated by reversed-phase high-performance liquid chromatography (HPLC) (*12*) and the recovered proteins tested in the acute infectivity assay with HIV-1_{IIIB}. Fractions containing suppressive activity were subjected to additional rounds of reversed-phase HPLC. Suppressive activity against HIV-1_{IIIB} in the absence of cytotoxic effects consistently copurified with a single protein peak that appeared as a homogeneous 8-kD band when analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) (Fig. 1). This protein was not reactive in enzyme-linked immunosorbent assays (ELISAs) for RANTES, MIP-1α, or MIP-1β (R&D Systems, Minneapolis, Minnesota). Amino-terminal sequence analysis of the purified protein yielded the sequence YGANMEDSV**RDYVRYRL; a minor sequence, PYGANME, was also obtained (*15*). After assigning the ambiguous cycles (*) to cysteine residues, a comparison of these sequences with known chemokines revealed identity with the recently described β-chemokine macrophage-derived chemokine (MDC) (*16*). We did not detect a peptide sequence beginning with glycine, in con-

Table 1. Production of HIV-1–suppressor activity and β-chemokines by HTLV-1–transformed CD8$^+$ T Cells from HIV-1–infected individuals. Cell-free supernatants from immortalized CD8$^+$ T cell lines were tested with primary NSI and SI isolates and the TCLA isolate HIV-1_{IIIB} (*7*). Chemokines released into the supernatants were assayed by ELISA (R&D Systems). The concentrations of HIV-1 p24 in control assays without test supernatants were 211.56, 290, and 300.26 ng/ml for HIV-1_{IIIB}, NSI, and SI viruses, respectively. Data are means of duplicate assays.

Clone	Inhibition of HIV-1 infection (%)			Chemokine (ng/ml)		
	SI 06	NSI 15	IIIB	RANTES	MIP-1α	MIP-1β
F3b clone 3	0	99.9	0	137.1	78	>100
F3b clone 19	88.8	99.9	53.7	68.3	35	21.4
A2a clone 2	12.75	74.24	56.7	115	35	9.6
B3b clone 2	42.8	87.3	0	83	23.8	5.4
B2 clone 12	0	94.29	0	56	65	8
A2 clone 5	50.07	99.8	0	109.8	53	25.2

trast to the NH_2-terminal sequence obtained for MDC produced in CHO cells (*16*). This difference is probably due to variability in NH_2-terminal processing between cell types; such variable processing occurs with other chemokines (*17*) and is associated with increased potency (*18*).

A reversed-phase HPLC fraction (fraction 27) containing native MDC (nMDC) purified from F3b clone 19 cell cultures suppressed the acute infection of $CD8^+$ cell–depleted PBMCs by HIV-1_{IIIB} in a concentration-dependent manner (Fig. 2). In contrast, flanking fractions (fractions 26 and 28) not containing nMDC had no effect. Two preparations of purified nMDC (MDC 1 and MDC 2) were further tested with a variety of primary NSI isolates in the acute infectivity assay with $CD8^+$ cell–depleted PBMCs as well as in infectivity assays with PM1 target cells and the primary macrophage-tropic isolate HIV-1_{BaL}. Purified nMDC markedly suppressed all of the NSI isolates tested (Table 2). However, MDC 2 did not suppress the infection of PM1 cells by HIV-1_{BaL}, even though an HPLC fraction containing RANTES produced the expected suppressive effect. In contrast, suppression of HIV-1_{BaL} by nMDC (preparation MDC 3) was observed with PBMCs as target cells, indicating that target cell type is an important determinant of activity. Further experiments revealed that nMDC was also able to suppress primary simian immunodeficiency virus (SIV) strain mac_{251}, HIV-1 isolate SI 06, and another primary SI isolate, 22069-04 (*7*). Therefore, suppression by MDC is not restricted to HIV-1 or to a specific viral phenotype.

The ability of nMDC to suppress HIV-1 in the acute infectivity assay suggested that activated $CD8^+$ cell–depleted T cells express the MDC receptor. MDC induces the migration of monocytes, activated natural killer cells, and monocyte-derived dendritic cells (*16*), but its effects on T cells are less well characterized. Chemokines stimulate a rapid and transient increase in cytosolic Ca^{2+} concentration (*19*) coincident with the induction of a chemotactic effect. To verify that HIV-1 target cells express an MDC receptor, we investigated the effects of purified nMDC on the cytosolic free Ca^{2+} concentration in activated $CD8^+$ cell–depleted and unfractionated PBMCs by flow cytometry (*20*). In both instances, treatment with 3 nM nMDC induced a transient increase in intracellular Ca^{2+} concentration (Fig. 3). Control experiments with 3 nM stromal cell–derived factor–1β (SDF-1β) or RANTES (R&D Systems) produced the expected marked increase in intracellular Ca^{2+} concentration in the $CD8^+$ cell–depleted and unfractionated PBMCs, respectively. Together with the suppressive effect on T cell–tropic isolates, these results indicate that an MDC receptor is expressed on the target cells used in the infectivity assay.

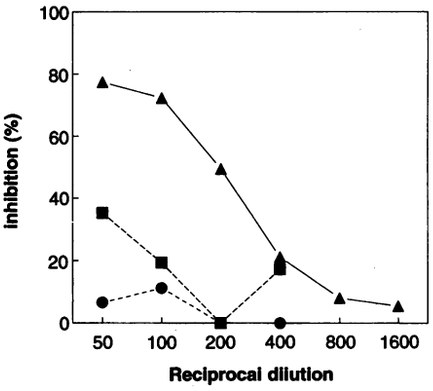

Fig. 2. HIV-1–suppressive activity of purified nMDC. PBMCs (2×10^5) from normal donors were activated with PHA and depleted of $CD8^+$ cells (*7*) and then exposed to 50 $TCID_{50}$ of HIV-1_{IIIB} for 3 hours at 37°C. The cells were then washed and treated with various dilutions of reversed-phase HPLC fraction 27 containing purified MDC (▲) or of flanking fractions 26 (●) or 28 (■). After 2 days, cultures were replenished with fresh medium containing the corresponding dilution of the respective fraction. Virus replication was determined by HIV-1 p24 ELISA of the culture medium on day 5. Percentage of inhibition of infection was calculated relative to the extent of infection in control assays performed in the absence of test sample. The concentration of MDC present in fraction 27 was 10 μg/ml as determined by amino acid analysis. Data are from a typical experiment repeated four times with similar results.

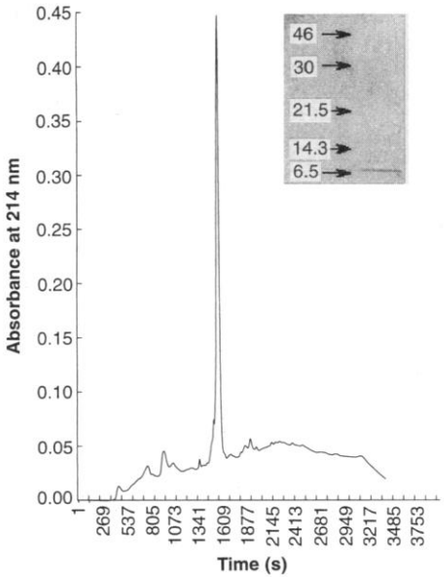

Fig. 1. Reversed-phase HPLC profile of MDC purified from serum-free culture supernatant of F3b clone 19 cells. **(Inset)** A portion of the peak fraction was analyzed by SDS-PAGE and stained with Coomassie blue. The positions of molecular size standards are shown in kilodaltons (arrows).

Table 2. Effects of purified nMDC on infection by HIV-1 isolates. Two preparations of purified nMDC (MDC 1 and MDC 2) were tested for suppressor activity with a panel of primary NSI isolates (*7*) and the TCLA isolate HIV-1_{IIIB}. NSI 03 and NSI 15 were obtained sequentially from the same individual (*7*). MDC 1 (fraction 27) was tested at 200 ng/ml. MDC 2 was tested at a dilution (1:30) that produced an equivalent concentration of chemokine, as determined by peak area on the reversed-phase HPLC chromatogram, compared with that obtained for fraction 27. The assays with MDC 1 were performed in 96-well microtiter plates in a total volume of 200 μl to conserve material. The medium was replenished on day 3 by removing 100 μl of the old medium and replacing it with fresh medium containing MDC 1. The assays were otherwise performed as described (*7*). MDC 2 was also tested in an acute infectivity assay with PM1 cells and primary HIV-1_{BaL}. PM1 cells (1×10^5) were infected with 100 $TCID_{50}$ of virus (propagated in primary macrophages) for 6 hours at 37°C. Cells were washed and treated with purified nMDC, and, after 2 days, the medium was replenished with fresh medium containing chemokine. RANTES (25 ng/ml) purified from the F3b clone 19 culture supernatant was assayed in parallel as a control. In a separate series of experiments, another preparation of nMDC (MDC 3) was tested at a 1:30 dilution for suppression of primary SIV_{mac251}, HIV-1_{BaL}, and primary SI isolates with PBMCs as target cells (*7*). HIV-1_{IIIB} was also tested for comparison. Assays with SIV_{mac251} (propagated and titered in rhesus monkey PBMCs) were modified to use 500 $TCID_{50}$ of virus per 1×10^6 cells. Virus replication was determined by p24 ELISA of the culture supernatant on days 4 to 6. Percentage of inhibition of infection was calculated relative to the extent of infection in control assays performed in the absence of chemokine. Data are means of triplicate assays. NT, not tested.

Protein	Inhibition of infection (%)				
	NSI 22069-03	NSI 03	NSI 15	IIIB	PM1/BaL
MDC 1	98.7	NT	NT	77.4	NT
MDC 2	80.51	91.46	88.15	82.7	0
RANTES	NT	NT	NT	8.5	99.50
	SI 22069-04	SI 06	SIV_{mac251}	IIIB	PBMC/BaL
MDC 3	81	83	66.3	68.7	54.7

The expression of MDC in primary PBMCs and in PM1 and HUT 78 T cell lines was evaluated by Northern (RNA) blot analysis (*21*). An intense signal of the expected size (~3 kb) was detected with RNA from F3b clone 19 cells, whereas no signal was detected with RNA from PM1 or HUT 78 cells in the presence or absence of interleukin-2 (IL-2) (Fig. 4). Similarly, no signal was detected with primary resting PBMCs from a healthy donor. However, a marked hybridization signal was detected with RNA from the PBMCs 48 hours after activation with PHA and recombinant human IL-2. These results differ from those of a previous study that failed to detect MDC expression in PBMCs (*16*). However, it is likely that MDC expression is variable and becomes marked under the activation and culture conditions used here.

Northern blot analysis of F3b clone 3, B3b clone 2, and A2 clone 5 cells revealed MDC signals equivalent to that detected with F3b clone 19 (*22*). Thus, the ability of F3b clone 19 culture supernatant to suppress HIV-1_{IIIB} is not due to differential MDC gene expression and may instead be related to posttranslational processes that result in enhanced secretion or activity of the protein. It is also possible that the overall suppressive activity produced by the clones is determined by a combination of MDC and other molecules that either enhance or inhibit suppressive effects.

To date, the known suppressive chemokines are ligands for receptors used by HIV-1 as coreceptors for virus entry (*9*, *23*). The viral phenotype determines which coreceptor is used for entry and, consequently, the chemokine that blocks infection. Thus, NSI viruses that require the CCR5 receptor are suppressed by RANTES, MIP-1α, and MIP-1β; SI viruses that use CXCR4 are suppressed by SDF-1 (*24*); and certain isolates that bind CCR3 are suppressed by eotaxin (*25*). Accordingly, the ability of MDC to suppress infection suggests the possibility that a putative MDC receptor serves as an additional coreceptor for certain NSI and SI strains of HIV-1. The capacity to use multiple coreceptors has already been demonstrated for several primary isolates (*8*, *26*). However, nMDC suppressed the isolates we tested even under conditions in which other functional coreceptors were likely to be present on the target cells. The reason for this effect is unclear, although it is possible that certain coreceptors, including an MDC receptor, act cooperatively in facilitating HIV-1 entry. Results similar to ours were obtained with microglia and reporter viruses containing NSI envelope sequences. In this instance, microglia expressing both CCR5 and CCR3 were protected from infection by either eotaxin or MIP-1β alone (*25*). Further experiments will be necessary to rule out the possibility that MDC might suppress infection by mechanisms that do not involve virus entry.

The assignment of suppressive activity to MDC should add new perspectives to the role of β-chemokines in the natural progression and prevention of HIV-1 infection. The presence of MDC receptors on both macrophages and dendritic cells has important implications for HIV pathogenesis, given that these cells are thought to be major vehicles for primary infection (*27*, *28*). MDC might inhibit the infection of dendritic cells, as has been shown for RANTES and SDF-1 (*28*). However, it is likely that the MDC receptor is not expressed on certain T cell lines, because MDC did not suppress HIV-1_{BaL} infection of PM1 cells. Clarification of these possibilities awaits a definition of the receptor (or receptors) for MDC.

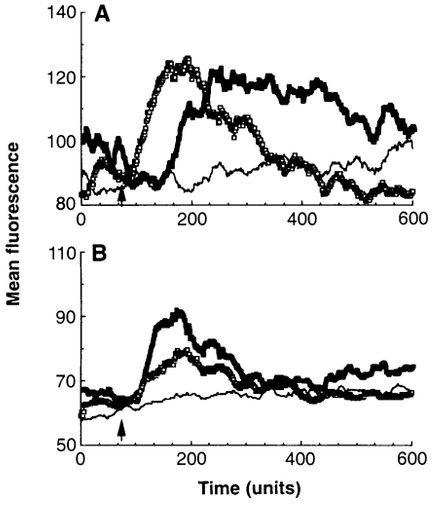

Fig. 3. Effects of MDC on intracellular Ca^{2+} concentration in $CD8^+$ cell–depleted and unfractionated PBMCs. (**A**) PBMCs were depleted of $CD8^+$ cells and cultured as described for the infectivity assay (*7*). The cells were stimulated with 3 nM SDF-1β (thick line) or nMDC purified by reversed-phase HPLC (□). (**B**) PBMCs were activated with PHA (5 μg/ml) and IL-2 (10 ng/ml) for 72 hours and then cultured in the presence of the same concentration of IL-2 for 14 days. The cells were then assayed as described (*20*) for changes in cytosolic Ca^{2+} concentration in response to 3 nM RANTES (thick line) or nMDC (□). In all experiments, control assays were performed with phosphate-buffered saline (thin lines). Chemokine or control additions were made at the time marked by the arrow. Data were acquired for at least 3 min. One time unit is equivalent to 0.2 s.

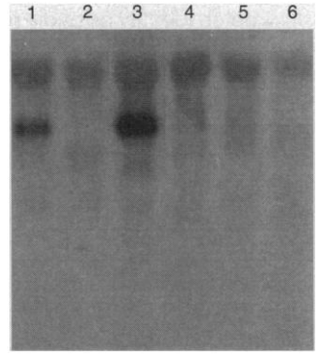

Fig. 4. Northern blot analysis of MDC mRNA in immortalized T cell lines and primary PBMCs. Northern blot analysis was performed as described (*21*). Lane 1, activated PBMCs; lane 2, resting PBMCs; lane 3, F3b clone 19; lane 4, HUT 78 plus IL-2; lane 5, HUT 78; and lane 6, PM1.

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6. $CD8^+$ T cell clones immortalized in vitro were prepared as previously described [P. D. Markham *et al.*, *Int. J. Cancer* **31**, 413 (1983); *ibid.* **33**, 13 (1984)]. Briefly, $CD8^+$ T cells were isolated from HIV-1–infected individuals by positive selection with magnetic beads coated with antibodies to CD8 (Dynal) and then activated with phytohemagglutinin (PHA) for 48 hours in complete medium (RPMI 1640 containing 15% fetal bovine serum, 1% glutamine, and 1% penicillin-streptomycin) supplemented with 10% IL-2. Cells were washed and exposed to HTLV-I by coculture at a ratio of 3:1 with an HTLV-I–producing T cell line that had been subjected to irradiation with 80 Gy for 30 min to prevent cell proliferation. Cultures were maintained until clusters of immortalized cells were observed (usually after 2 to 4 weeks of exposure to HTLV-I). The individual clusters were then transferred into separate culture flasks. Rapidly proliferating cells were subjected to single-cell cloning by limiting dilution and then expanded in complete medium containing 10% IL-2. A $CD8^+$ phenotype of the immortalized cell lines was verified by flow cytometry with antibodies to CD8 and to CD4 (Becton-Dickinson, San Jose, CA).
7. PBMCs from normal donors were activated with PHA for 48 hours and depleted of $CD8^+$ T cells by negative selection with magnetic beads coated with antibodies to CD8 (Dynal). After culture for 18 hours in complete medium containing recombinant human IL-2 (rIL-2) (Gemini Biotech, Woodland, TX) at 16 ng/ml, the cells (1×10^6) were exposed to 250 $TCID_{50}$ (median tissue culture infectious dose) of the indicated HIV-1 isolates for 3 hours at 37°C. Cells were then washed and suspended in complete medium with rIL-2 and placed into 48-well plates (2×10^5 cells per well) with a 1:4 dilution of culture supernatant (or the dilution of chemokine indicated in other experiments) in a total volume of 250 μl. Control infections were performed in complete medium with rIL-2 alone. After 48 hours, the medium in each well was replenished with 250 μl of fresh medium containing the same culture supernatant (or chemokine). The extent of infection was measured on either

day 5 or 6 with an HIV-1 p24 ELISA (Organon Teknika, Durham, NC). The suppressive activity of each supernatant was calculated as the percentage of inhibition of HIV-1 p24 antigen production compared with controls. HIV-1_{IIIB} virus stock was prepared from chronically infected Molt3–HIV-1_{IIIB} cell lines, whereas the previously described primary NSI and SI isolates (*8*) [R. I. Connor *et al.*, *J. Virol.* **67**, 1772 (1993)] were propagated in primary PBMCs. All isolates were titrated to determine $TCID_{50}$ in PHA-stimulated normal PBMCs.

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11. F3B clone 19 cells were grown in complete medium containing rIL-2 (16 ng/ml) at 37°C in a CO_2 incubator. After expanding the culture to 200 ml, the cells were isolated by centrifugation and resuspended in RPMI medium containing HB101 (Irvine Scientific, Santa Ana, CA) and supplemented with rIL-2 (16 ng/ml), 1% glutamine, and 1% penicillin-streptomycin. The cells were grown to confluence, and the medium was then harvested by centrifugation at 670*g* for 10 min.
12. Culture supernatant (1200 ml) from F3b clone 19, grown to high cell density in serum-free medium supplemented with rIL-2 (*11*), was subjected to centrifugation at 100,000*g* for 60 min at 4°C, and the resulting soluble fraction was applied to a 5-ml Hi-Trap heparin affinity FPLC (fast protein liquid chromatography) column (Pharmacia) that had been equilibrated with 10 mM tris-HCl (pH 7.6) containing 0.1 M NaCl (column buffer). The column was then washed extensively with column buffer, after which the bound proteins were eluted with 10 mM tris-HCl (pH 7.6) containing 2.0 M NaCl at a flow rate of 1 ml/min. The column eluate was adjusted to pH 2.0 by addition of trifluoroacetic acid (TFA) and subjected to reversed-phase HPLC on a PEEK C_{18} column (Waters Instruments, Millford, MA) that had been equilibrated with H_2O containing 0.1% TFA. Proteins bound to the column were eluted with a 5-min linear gradient of aqueous acetonitrile (0 to 35%) containing 0.1% TFA. After 10 min at 35% acetonitrile, the column was further subjected to a 60-min linear gradient of 35 to 70% aqueous acetonitrile containing 0.1% TFA. The flow rate was maintained at 0.5 to 1 ml/min. The resulting fractions were tested for suppressor activity in the infectivity assay with HIV-1_{IIIB}. Active fractions were pooled, diluted twofold in H_2O containing 0.1% TFA, and reapplied to the column. Proteins were eluted with a 30-min linear gradient of aqueous acetonitrile (0 to 60%) containing 0.1% TFA at a flow rate of 0.5 to 1 ml/min. The fractions obtained were assayed as above. Active fractions were pooled, diluted with H_2O containing 0.1% TFA, and fractionated under the same conditions to obtain a single protein peak. The fraction corresponding to the peak and flanking fractions were tested in the infectivity assay to verify that suppressor activity co-fractionated with the protein.
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15. Amino acid analysis of the purified protein was performed with a Beckman 6300 amino acid analyzer. Samples were hydrolyzed for 24 hours at 110°C in the presence of 6 M HCl and then reconstituted in loading buffer. Amino acids were analyzed by post-column derivatization with ninhydrin. NH_2-terminal microsequencing was performed by automated Edman degradation with a Hewlett-Packard G1005A protein sequencing system, which was operated with standard reagents, solvents, and programs (routine 3.0) supplied by the manufacturer. Abbreviations for the amino acid residues are A, Ala; D, Asp; E, Glu; G, Gly; L, Leu; M, Met; N, Asn; P, Pro; R, Arg; S, Ser; V, Val; and Y, Tyr.
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20. Intracellular Ca^{2+} was measured by flow cytometry according to a modification (J. Burns and G. Lewis, *Biotechniques*, in press) of previously described methods [R. Badolato *et al.*, *J. Immunol.* **155**, 4004 (1995); R. Greimers *et al.*, *Cytometry* **23**, 205 (1996)]. Briefly, unfractionated or $CD8^+$ cell–depleted PBMCs (1×10^6 cells/ml) prepared as described (*7*) were cultured in the absence of IL-2 for 1 hour, washed with RPMI 1640 (GIBCO BRL) containing 25 mM Hepes but no phenol red or sodium bicarbonate, and resuspended in the same solution at a density of 2×10^7 cells/ml. Cells (1×10^6) were then added to sample tubes, loaded for 20 min at 37°C with 2 μM fluo-3 (Molecular Probes) reconstituted in a solution containing 20% Pluronic F-127 (Molecular Probes) and dimethyl sulfoxide, and stained with 7-aminoactinomycin D (Molecular Probes) to discriminate dead cells [I. Schmid *et al.*, *Cytometry* **13**, 204 (1992)]. The samples were then washed once as before and resuspended in 1 ml of RPMI 1640 without phenol red and sodium bicarbonate. All samples were maintained at 20°C in the dark until 5 min before analysis, at which time the sample tube was placed in a 37°C water bath. Cells were maintained at 37°C throughout data acquisition. Cells were stimulated by addition of test chemokine to a final concentration of 3 nM. Data were acquired with a FACSCalibur (Becton-Dickinson) flow cytometer, with excitation at 488 nm. Cells were gated by forward- and side-scatter properties as well as by exclusion of 7-aminoactinomycin D fluorescence by use of emission above 650 nm in the FL-3 window. Calcium mobilization was determined by a two-parameter density plot of linear emissions collected at 530 nm in the FL-1 window for the gated cell population over time.
21. PBMCs (American Red Cross) from a healthy donor were purified by centrifugation in Histopaque (Sigma) and harvested either immediately or after activation for 48 hours with PHA (5 μg/ml) and rIL-2 (10 ng/ml). The HUT 78 human T cell line was cultured in the presence (50 U/ml) or absence of IL-2 (Boehringer, Mannheim, Germany). RNA was isolated by the RNAzol procedure (Tel-Test, Friendswood, TX), and 10 μg of total cellular RNA was separated by electrophoresis on a denaturing formaldehyde-agarose gel and then transferred to a nylon membrane. The membrane was subjected to hybridization with an MDC-specific probe and washed under stringent conditions as described [A. Garzino-Demo, R. C. Gallo, S. K. Arya, *Hum. Gene Ther.* **6**, 177 (1995)]. The probe for Northern hybridizations was generated by reverse transcription and polymerase chain reaction with MDC-specific primers.
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Fyn-Kinase as a Determinant of Ethanol Sensitivity: Relation to NMDA-Receptor Function

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Animals vary in their sensitivity to ethanol, a trait at least partly determined by genetic factors. In order to identify possible responsible genes, mice lacking Fyn, a non–receptor type tyrosine kinase, were investigated. These mice were hypersensitive to the hypnotic effect of ethanol. The administration of ethanol enhanced tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor (NMDAR) in the hippocampus of control mice but not in Fyn-deficient mice. An acute tolerance to ethanol inhibition of NMDAR-mediated excitatory postsynaptic potentials in hippocampal slices developed in control mice but not in Fyn-deficient mice. These results indicate that Fyn affects behavioral, biochemical, and physiological responses to ethanol.

Ethanol (EtOH) is among the most widely abused drugs in the world, yet the neural mechanisms responsible for EtOH intoxication and dependence are largely unknown. Genetic factors affect the determination of the behavioral responses to EtOH in rodents and humans (*1*), but few specific genes that increase or decrease the drug actions have been reported (*2*, *3*).

Tyrosine kinases phosphorylate *N*-methyl-D-aspartate (NMDA) and γ-aminobutyric acid A ($GABA_A$) receptors and modulate the electrophysiological function of these receptors (*4*–*6*). The function of these receptors is also modulated by EtOH, and they are hypothesized to be targets