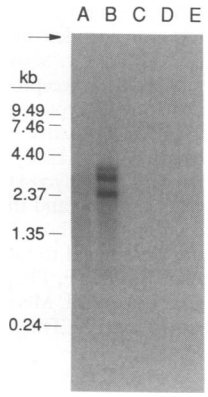

Fig. 4. RNA hybridization on blots with a cDNA probe from the human IL-8 receptor. Lane A, 5 μg of RNA not polyadenylated from human neutrophils; B, 2 μg of polyadenylated [poly(A)$^+$] RNA from human neutrophils; C, 2 μg of poly(A)$^+$ RNA from U937 cells; D, 2 μg of poly(A)$^+$ RNA from U266 cells; E, 2 μg of poly(A)$^+$ RNA from Jurkat cells. RNA was separated by size by electrophoresis on a formaldehyde (1%) gel (*25*), transferred to nitrocellulose, hybridized to the full-length cDNA insert from clone pRK5B.il8r1.1, and washed in 30 mM sodium chloride, 3 mM trisodium citrate at 55°C.

was detected to mRNA from U266 or Jurkat cell lines, which are of the B cell and T cell lineages (Fig. 4). IL-8 does not bind to cells from these lineages (*11*). No hybridization to mRNA from the monocyte cell line U937 was detected, although low levels of IL-8 binding to these cells has been reported (*11*).

Alignment of the sequences of the human receptors for the three neutrophil chemoattractants IL-8, fMLP (*15*), and C5a (*19*) shows the similarity (29 to 34% amino acid identity) of these G protein–coupled receptors (Fig. 2). The third intracellular loop of receptors in this subfamily is shorter than that in other G protein–coupled receptors such as the β-adrenergic (*4*) or muscarinic acetylcholine receptors (*20*). This loop contains determinants at least partially responsible for the binding of G proteins to the receptors (*4*). The intracellular COOH-terminal region of the IL-8 receptor has little similarity to those of the fMLP and C5a receptors but it does contain several serine and threonine residues that may function as phosphorylation sites. Like the C5a receptor (*19*), the NH_2-terminal extracellular region of the IL-8 receptor has several acidic residues. These amino acids may participate in the binding of IL-8, which is quite basic (pI ~9.5), to the receptor. The amino acid sequence presented here is 77% identical with a receptor sequence presented in the accompanying paper (*21*) including consecutive amino acids matches of 105 and 64 amino acids. These two sequences may be members of a family of related receptors for the IL-8 family of cytokines.

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Cloning of Complementary DNA Encoding a Functional Human Interleukin-8 Receptor

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Interleukin-8 (IL-8) is an inflammatory cytokine that activates neutrophil chemotaxis, degranulation, and the respiratory burst. Neutrophils express receptors for IL-8 that are coupled to guanine nucleotide-binding proteins (G proteins); binding of IL-8 to its receptor induces the mobilization of intracellular calcium stores. A cDNA clone from HL-60 neutrophils, designated p2, has now been isolated that encodes a human IL-8 receptor. When p2 is expressed in oocytes from *Xenopus laevis*, the oocytes bind ^{125}I-labeled IL-8 specifically and respond to IL-8 by mobilizing calcium stores with an EC_{50} of 20 nM. This IL-8 receptor has 77% amino acid identity with a second human neutrophil receptor isotype that binds IL-8 with higher affinity. It also exhibits 69% amino acid identity with a protein reported to be an N-formyl peptide receptor from rabbit neutrophils, but less than 30% identity with all other known G protein–coupled receptors, including the human N-formyl peptide receptor.

IL-8, ALSO KNOWN AS NEUTROPHIL ACTIVATING PROTEIN–1 or NAP-1, is a potent chemoattractant for neutrophils that is produced by many cell types in response to inflammatory stimuli (*1*). IL-8 is structurally and functionally related to several members of the macrophage inflammatory protein–2 (or MIP-2) family of cytokines. These include MIP-2, MGSA (melanoma growth-stimulating activity), and NAP-2 (*2–4*). High affinity binding sites for IL-8 have been found on transformed myeloid precursor cells such as HL-60 and THP-1 as well as on neutrophils (*5, 6*). NAP-2 and MGSA compete with IL-8

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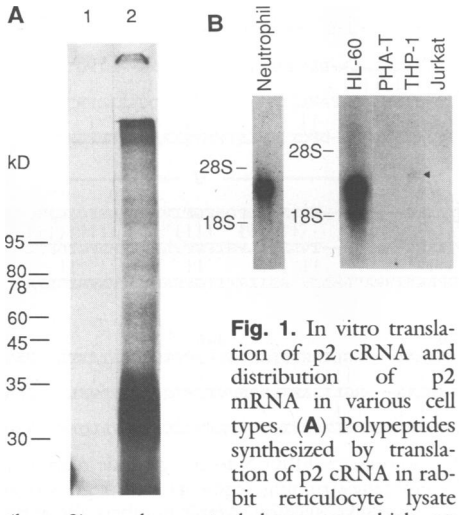

Fig. 1. In vitro translation of p2 cRNA and distribution of p2 mRNA in various cell types. (**A**) Polypeptides synthesized by translation of p2 cRNA in rabbit reticulocyte lysate (lane 2) or by control lysate to which no RNA was added (lane 1). Red blood cell membrane proteins were used as molecular size standards indicated in kilodaltons (kD) at the left. The gel was exposed to XAR-2 film for 12 hours (*14*). (**B**) Distribution of p2 mRNA. The blot of RNA from neutrophils was prepared separately from 10 μg of total cellular RNA. The other lanes derive from a single blot containing total cellular RNA from peripheral blood T lymphocytes activated with phytohemagglutinin (PHA-T, 5 μg), THP-1 cells (5 μg), and Jurkat cells (3 μg). The lane marked HL-60 contains 10 μg of polyadenylated [poly(A)$^+$] RNA from undifferentiated HL-60 cells. The arrow indicates the location of a faint band of RNA from THP-1 cells. Both blots were hybridized under identical conditions with the same p2 probe and were washed at 68°C in 0.1× SSPE (*12*) for 1 hour. Blots were exposed to XAR-2 film in a Quanta III cassette at −80°C for 5 days. Results from three independent HL-60 cell preps and two separate THP-1 and neutrophil blots were identical.

for binding to human neutrophils, suggesting that they interact with the same receptors (*5*). Stimulation of neutrophils with IL-8, NAP-2, or MGSA causes mobilization of intracellular calcium stores and elicits motile, secretory, and metabolic responses that are critical to the role of the neutrophil in host defenses (*3, 4, 7*).

Functional expression in the *Xenopus* oocyte has established the identity of cDNA clones encoding rabbit (*8*) and human (*9, 10*) forms of another peptide chemoattractant receptor on neutrophils, the N-formyl peptide receptor. Yet the amino acid sequence of the rabbit form of the receptor (originally designated F3R) is only 28% identical with that of the human form (designated in this paper as FPR); this dissimilarity is far greater than the differences between species reported for all other G protein–coupled receptors (*11*).

We therefore attempted to identify a human homolog of F3R. An oligonucleotide probe corresponding to nucleotides 238 to 276 of the cDNA sequence of F3R (*8*) was hybridized to cDNA libraries made from RNA from the promyelocytic leukemia cell line HL-60 grown for 2 days in the presence of dibutyryl cyclic adenosine monophosphate (750 μM), a treatment that induces a neutrophil-like phenotype. Seven clones that encoded an identical gene product were isolated (*12*). The longest of these, designated p2, was sequenced on both strands. Confirmatory sequences were obtained from the other clones. A 1065-bp open reading frame begins with the sequence AACATGG, which conforms to the Kozak consensus criteria for translation initiation sites (*13*). A 24-bp polyadenylate tail is found at the end of a 405-bp 3′ untranslated region.

RNA synthesized in vitro from p2 cDNA (p2 cRNA) directed the synthesis of a polypeptide of 32 kD in vitro (Fig. 1A) (*14*). This is the size of the deglycosylated native N-formyl peptide receptor (*15*) and the FPR protein synthesized in vitro (*16*). Binding sites for N-formyl peptides are expressed in mature, but not in immature, myeloid cells (*17*). Expression of RNA for FPR is also restricted to mature myeloid cells (*10*). In contrast, a p2 probe hybridized with a single 3-kb band on blots of RNA from the myeloid precursor cell lines HL-60 and THP-1, and from normal blood-derived human neutrophils but did not hybridize with RNA from peripheral blood T lymphocytes or Jurkat cells (Fig. 1B) (*18*). This pattern of expression of p2 RNA is more like the distribution of IL-8 binding sites than that of N-formyl peptide binding sites (*6*).

Xenopus oocytes that had been injected with p2 cRNA mobilized intracellular calcium in response to IL-8 with a mean effective concentration (EC_{50}) of 20 nM (Fig. 2A), (*19*) but did not respond to N-formyl methionyl-leucyl-phenylalanine (fMLP). This value is approximately 20-fold higher than that reported for stimulation of human neutrophils with recombinant human IL-8 (*3*). The receptor specifically bound IL-8 over the same concentration range as that required for stimulation of calcium flux (Fig. 2B) (*20*). Because specific binding was not saturated at the highest concentration of radioligand tested, a dissociation constant could not be determined. Thus, the receptor encoded by p2 is a low affinity IL-8 receptor not previously recognized on human neutrophils (*5, 6*). The receptor also activated a calcium flux in response to structurally related ligands with the following order of potency: IL-8 > MGSA > NAP-2 (Fig. 2C), which correlates with the effectiveness of these compounds in competing with ^{125}I-labeled IL-8 for binding to neutrophils (*5*). C5a, a structurally unrelated chemoattractant that is similar in size (74 amino acids) and charge (pI 8.6) to IL-8, did not activate the IL-8 receptor (Fig. 2C).

The IL-8 receptor contains seven hydrophobic segments predicted to span the cell membrane, a characteristic of the superfamily of G protein–coupled receptors (Fig. 3). The COOH-terminal segment contains 11 serine or threonine residues that may be phosphorylation sites for cellular kinases. The third cytoplasmic loop, which may interact with G proteins, consists of 20 amino acids, similar in size to that of other peptide receptors. The IL-8 receptor has a single predicted site for N-linked glycosylation in

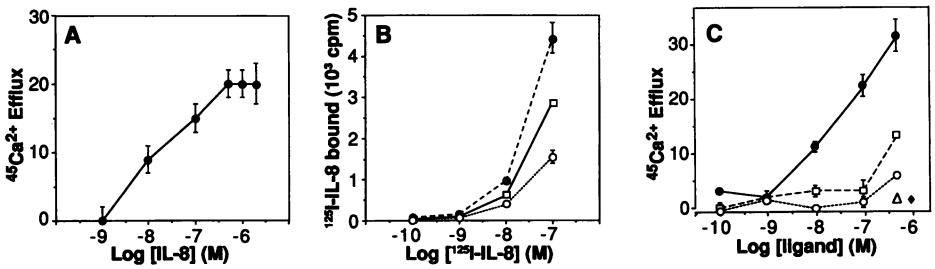

Fig. 2. Expression of a human IL-8 receptor in *Xenopus* oocytes. (**A**) Signal transduction by the IL-8 receptor. Four days after injection with 5 ng of p2 cRNA, oocytes were stimulated with the indicated concentration of IL-8 and Ca^{2+} efflux was measured. The data are derived from five replicate determinations per point and are representative of three separate experiments. (**B**) Binding of ^{125}I-labeled IL-8 to oocytes expressing a functional IL-8 receptor. Total (●) and non–specific binding (○) was determined by incubating oocytes injected with p2 cRNA with the indicated concentration of ^{125}I-labeled IL-8 in the absence or presence of unlabeled IL-8 (1 μM), respectively. Data are the mean ± SEM of triplicate determinations per point and are representative of two separate experiments. Non–specific binding was subtracted from total binding to determine specific binding (□). C5a (1 μM) did not displace ^{125}I-labeled IL-8 from oocytes injected with p2 cRNA. Specific binding of ^{125}I-labeled IL-8 by oocytes injected with water was undetectable. (**C**) Ligand selectivity of the IL-8 receptor. Three days after injection with 5 ng of p2 cRNA, oocytes were stimulated with the indicated concentration of IL-8 (●), MGSA (□), NAP-2 (○), fMLP (◆), or C5a (△) and Ca^{2+} efflux activity was measured. The data are derived from eight replicate determinations per point. The percent of total $^{45}Ca^{2+}$ released from oocytes injected with 50 ng of HL-60 neutrophil RNA in response to fMLP (1 μM) or C5a (500 nM) was 51 ± 3 and 16 ± 5%, respectively. The response of oocytes injected with 5 ng of an irrelevant cRNA encoding the rat serotonin 1c receptor (*10, 25*) was negligible for each of the five ligands; the response to the relevant ligand, serotonin (1 μM), was 34 ± 3% (n = 6). In (A) and (C) basal amounts of calcium efflux and calcium uptake were similar among all experimental conditions.

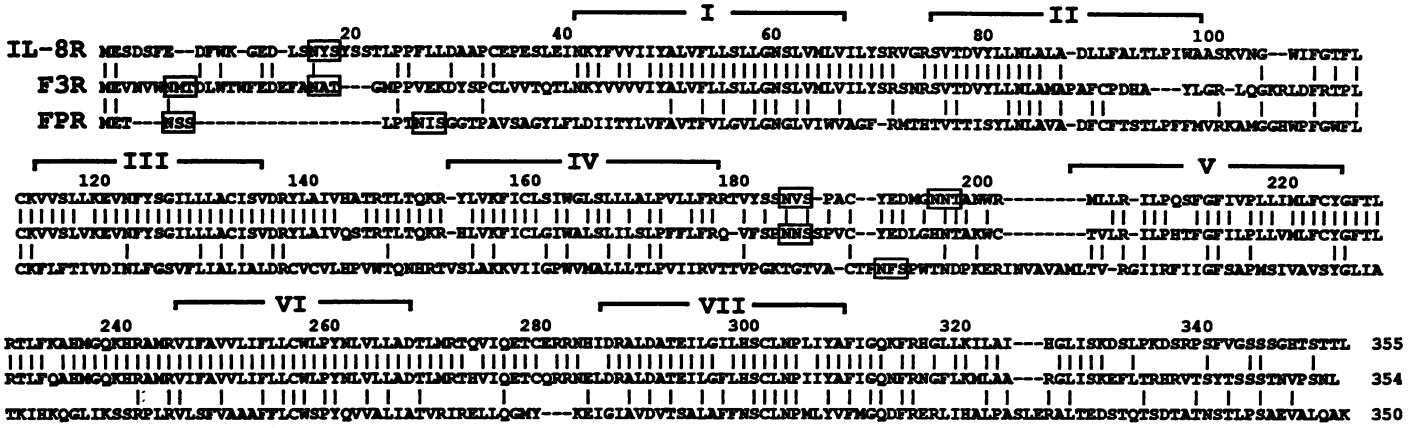

Fig. 3. Primary structure of a human IL-8 receptor (IL-8R) and alignment with that of the rabbit (F3R) and human (FPR) N-formyl peptide receptors. Vertical bars indicate identical residues for each adjacent sequence position. The location of predicted membrane-spanning segments I through VII as determined by the Kyte-Doolittle algorithm (*26*) are indicated. Open boxes designate predicted sites for N-linked glycosylation. Arabic numbers above the sequence blocks refer to the IL-8 receptor sequence and are left justified. Dashes indicate gaps that were inserted to optimize the alignment. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The nucleotide sequence has been deposited at GenBank (accession number M73969).

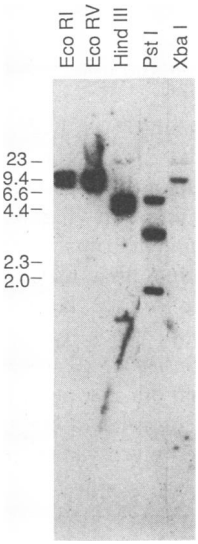

Fig. 4. Genomic analysis of a human IL-8 receptor. A Nytran blot of human genomic DNA digested with the indicated restriction endonucleases was hybridized with full-length cDNA of the IL-8 receptor at high stringency (final wash at 68°C in 0.1× SSPE for 1 hour). The blot was exposed to Kodak XAR-2 film in a Quanta III cassette at −80°C for 5 days. The position of DNA size standards is indicated in kilobases at the left. The autoradiogram shown is representative of two independent experiments.

the NH_2-terminal segment and two sites in the second extracellular loop. As with the C5a receptor (*21*), the NH_2-terminal segment is rich in acidic residues and may form the binding site for IL-8, which is basic (pI ~9.5).

The IL-8 receptor possesses 69% amino acid identity to F3R after the imposition of ten gaps. If only the predicted transmembrane domains (TMD) are compared, 84% identity is found with F3R (*22*). Moderately conserved domains include the NH_2-terminal segment (38% identity, four gaps), the first extracellular loop (33%, one gap), and the COOH-terminal 23 residues (22%, no gaps). The third cytoplasmic loops are 95% identical. The IL-8 receptor shares less than 30% amino acid identity with all other reported G protein–coupled receptor sequences including that of FPR (Fig. 3).

When a p2 probe was hybridized under conditions of high stringency to blots of human genomic DNA (*23*), the banding pattern was most consistent with one copy per haploid genome of a small gene encoding the low affinity IL-8 receptor (Fig. 4). Detection of faint bands, however, in DNA digested with Eco RV, Hind III, and Xba I after long exposure of the blot suggested that a closer human homolog of F3R, and perhaps a high affinity IL-8 receptor, could be found with the p2 probe. We therefore rescreened cDNA libraries from HL-60 neutrophils with a p2 probe (*12*). The 13 hybridizing plaques were sequenced and all were identical to p2. Therefore, if a gene encoding a receptor more closely related to F3R is present in HL-60 cells, it must either not be expressed or be expressed in very low amounts.

In the accompanying paper, a cDNA from human neutrophils is described that encodes a high affinity IL-8 receptor (*24*). This receptor has 77% amino acid identity with the low affinity IL-8 receptor and is more closely related to F3R (79% versus 69% amino acid identity). Neither human IL-8 receptor interacts with N-formyl peptides. The low affinity form diverges most extensively from the other two sequences in the NH_2-terminal segment, although the acidic character of this region is conserved. Thus, the human neutrophil expresses at least two distinct calcium mobilizing IL-8 receptors. It is possible that the low affinity IL-8 receptor could be a high affinity receptor for an as yet undefined ligand similar in structure to IL-8. Structural comparison of the IL-8 receptors with F3R predicts that it encodes a high affinity rabbit IL-8 receptor.

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14. The p2 cRNA was synthesized by in vitro transcription with T3 RNA polymerase of a pBluescript construct that had been cleaved with Xho I. The p2 cRNA (500 ng) was incubated for 30 min at 30°C with rabbit reticulocyte lysate and [^{35}S]methionine (Promega, Madison, WI). Labeled proteins (40% of

the yield) were then separated by SDS–polyacrylamide gel electrophoresis [10% gels (Novex, Encinitas, CA)]. The gel was stained with Coomassie blue, fixed, impregnated with Fluoro-Hance (Research Products International, Mount Prospect, IL), dried, and subjected to autoradiography.
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19. The materials and methods used for the calcium efflux assay were as described [P. M. Murphy, E. K. Gallin, H. L. Tiffany, *J. Immunol.* **145**, 2227 (1990)]. Oocytes were microinjected with RNA samples in a total volume of 50 nl per oocyte 3 days after harvesting and were then incubated at 20°C to 23°C for 2 to 4 days. Oocytes were then incubated with $^{45}Ca^{2+}$ [50 μCi/ml (ICN Biomedicals, Costa Mesa, CA)] for 3 hours. After ten washes with medium, individual oocytes were stimulated with ligand in wells of a 96-well tissue culture plate containing 100 μl of medium. Three 100-μl samples of the incubation medium were collected and analyzed by liquid scintillation counting: (*a*) the final 100 μl wash (20 min) before application of ligand; (*b*) fluid containing the stimulus, removed after a 20-min incubation with the oocyte; and (*c*) the oocyte solubilized in SDS (1%) in medium 20 min after stimulation. Data are presented as the mean ± standard error of the mean (SEM) of the percent of loaded $^{45}Ca^{2+}$ that was released by individual oocytes in response to the stimulus, or $[(b - a) \div (b + c)] \times 100$. The fMLP and recombinant human C5a were from Sigma, St. Louis, MO. Recombinant human IL-8 was from Genzyme, Boston, MA. Recombinant human NAP-2 was from Bachem, Philadelphia, PA.
20. IL-8 was iodinated to a specific activity of 260 Ci/mmol as described (*15*). Single oocytes were incubated with ^{125}I-labeled IL-8 for 30 min on ice in 10 μl of binding buffer (Hanks balanced salt solution with 25 mM Hepes, 1% bovine serum albumin, pH 7.4). Unbound ligand was removed by centrifugation of the oocyte through 300 μl of F50 silicone fluid (General Electric, Waterford, NY). The tubes were quickly frozen and gamma emissions from the amputated tips were counted.
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22. Alignment with ten other G protein–coupled receptor sequences (*10, 11*) and examination of corresponding DNA sequences indicates that the apparent divergence of the IL-8 receptor from F3R between residues 92 and 105 is due to a frame shift in F3R.
23. Human genomic DNA (3 μg per lane) was digested with 6 units of Eco RI, Eco RV, Hind III, Pst I, or Xba I restriction endonucleases (Boehringer-Mannheim, Indianapolis, IN) and was then fractionated by electrophoresis on an agarose gel (1%). After denaturation in alkaline solution the DNA was transferred to a Nytran filter by capillary action.
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27. We thank H. Malech and J. Gallin for advice, support, and critical reading of the manuscript; C. Gerard for helpful discussions; R. Kenney for genomic DNA; D. Julius for serotonin 1c receptor cDNA; and P. Beckmann, S. Lyman, and D. Cerretti for the gift of recombinant human MGSA protein.

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Viral Persistence in Neurons Explained by Lack of Major Histocompatibility Class I Expression

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Viruses frequently persist in neurons, suggesting that these cells can evade immune surveillance. In a mouse model, 5×10^6 cytotoxic T lymphocytes (CTLs), specific for lymphocytic choriomeningitis virus (LCMV), did not lyse infected neurons or cause immunopathologic injury. In contrast, intracerebral injection of less than 10^3 CTL caused disease and death when viral antigens were expressed on leptomeningial and choroid plexus cells of the nervous system. The neuronal cell line OBL21 expresses little or no major histocompatibility (MHC) class I surface glycoproteins and when infected with LCMV, resisted lysis by virus-specific CTLs. Expression of MHC heavy chain messenger RNA was limited, but β_2-microglobulin messenger RNA and protein was made normally. OBL21 cells were made sensitive to CTL lysis by transfection with a fusion gene encoding another MHC class I molecule. Hence, neuronal cells probably evade immune surveillance by failing to express MHC class I molecules.

NEURONS ARE ESSENTIAL FOR RECEIVING, integrating, and passing information. Although they maintain many essential functions of an organism, they cannot be replaced once destroyed. Hence, it is likely that neurons have unique strategies to avoid injury. Neurons can be persistently infected by many viruses (*1*). The cardinal host response to eliminate virally infected cells is the generation of CTLs (*2*), which recognize and kill infected cells when viral peptides appear on the surface that are complexed to glycoproteins of the class I MHC (*3*). Yet, although activated CTLs can cross the blood brain barrier (*4*), they seem unable to lyse neurons that are persistently infected with any of several different RNA or DNA viruses.

Neurons may have evolved a selective survival mechanism to avoid CTL recognition and lysis, such as a failure to present viral peptides complexed by MHC glycoproteins on the cell surface. Although the consequence of escape from CTL-mediated lysis would be favorable to the neuron, it would allow viruses to persist in these cells.

Lymphocytic choriomeningitis virus (LCMV) infection is controlled primarily by virus specific CTLs (*2, 5*). We generated CTL clones to the major epitopes of LCMV (*6*): CTL clones 228 and 232 are D^b-restricted and recognize amino acid residues (aa) 278 to 286 of the LCMV glycoprotein (GP); CTL clones HD8, HD9, and HD47 are L^d-restricted and recognize aa 119 to 127 of LCMV nucleoprotein (NP); and CTL clone Q9 is H-2^q–restricted and sees aa 116 to 127 of NP (*6*). CTL clone K39 is H-2^k–restricted and recognizes a non-GP, non-NP epitope located on the L RNA strand of the virus [either the viral polymerase or Z protein (*6*)]. During acute LCMV infection of the brain, virus replication is restricted primarily to cells in the leptomeninges and choroid plexus and rarely, if ever, in neurons [(*7*) and Fig. 1, top]. During persistent infection, the opposite occurs: neurons are heavily infected, but few other nervous system cells express viral antigens (Fig. 1, bottom). We took advantage of such observations to transfer LCMV-specific CTL intracerebrally to mice either acutely (87 mice) or persistently (36 mice) infected with virus. Virally infected neurons evaded CTL-mediated injury, and persistently infected mice remained alive throughout the 2- to 4-week observation period, despite receiving doses of CTLs $>1 \times 10^6$ per mouse. In contrast, intracerebral transfer of similar CTL clones to acutely infected mice resulted in immunopathologic injury and death within 7 to 12 days. This phenomenon was MHC-restricted since CTL clones were effective only when injected into mice of matched MHC haplotypes, including H-2^b, H-2^d, H-2^k, and H-2^q (Fig. 1). Death correlated directly with the number of CTL transferred. The 50% lethal dose (LD_{50}) end point ranged from 2×10^2 to 8×10^2 CTL for all haplotypes (tenfold dilutions of CTL from 10^6 to 10^1, six mice per group). Thus, although CTLs efficiently lysed virus-infected cells of the choroid plexus and leptomeninges, which express MHC class I glycoproteins, they did not kill neurons infected with virus.

To examine the mechanism by which neurons escape CTL lysis, we studied interactions between neurons and CTL in vitro. The OBL21 cell line was established by in vitro transformation of olfactory bulb cells of newborn CD1 mice (H-2^q haplotype) with a retroviral vector (*8*). Such cells re-

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