Brief Definitive Report

Intracerebral Injection of Proinflammatory Cytokines or Leukocyte Chemotaxins Induces Minimal Myelomonocytic Cell Recruitment to the Parenchyma of the Central Nervous System

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Summary

Neither excitotoxic neurodegeneration nor lipopolysaccharide induces an acute myelomonocytic exudate in the murine central nervous system (CNS) parenchyma (Andersson, P.-B., V. H. Perry, and S. Gordon. 1991. *Neuroscience.* 42:201; Andersson, P.-B., V. H. Perry, and S. Gordon. 1992. *Neuroscience.* 48:169). In this study formyl-methionyl-leucyl-phenylalanine, platelet-activating factor, interleukin 8 (IL-8), IL-1, or tumor necrosis factor α were injected into the hippocampus to assess whether these leukocyte chemotaxins and known mediators of recruitment could bypass this block. They induced morphologic activation of microglia and widespread leukocyte margination but little or no cell exudation into the CNS parenchyma. By contrast, there was acute myelomonocytic cell recruitment to the choroid plexus, meninges, and ventricular system, comparable to that in the skin after subcutaneous injection. The normal CNS parenchyma appears to be a tissue unique in its resistance to leukocyte diapedesis, which is shown here to be at a step beyond chemotactic cytokine secretion or induction of leukocyte adhesion to cerebral endothelium.

Our earlier studies have drawn attention to the fact that leukocyte responses after acute injury to the central nervous system (CNS) parenchyma (i.e., the grey and white matter) are not typical of that found in the other body tissues where rapid recruitment of neutrophils and monocytes is a consistent phenomenon (1–6). The myelomonocytic leukocyte infiltrate found in the degenerating distal segment of the optic nerve after a crush injury is less than that found in sciatic nerve (7). Since Wallerian degeneration is slower in CNS axons (8), crush injury in the optic nerve may provide a weak proinflammatory stimulus and thus could account for the difference in leukocyte responses. To test this, we induced rapid synchronous neuronal degeneration in the hippocampus with the excitotoxin kainic acid. Notwithstanding extensive acute neuronal necrosis and breakdown of the blood-brain barrier, there was no neutrophil recruitment and a 2-d delay before macrophage-microglial numbers increased in the CNS parenchyma (9). Kainic acid itself did not induce acute inflammation in non-CNS tissue, and thus in order to compare leukocyte responses in CNS parenchyma with that in other organs, we injected the potent proinflammogen LPS directly into the hippocampus. The injection of 20 ng failed to induce any leukocyte recruitment, and doses up to 2 μg induced only minimal acute neutrophil exudation and a 2-d delay before a phase of monocyte-restricted recruitment commenced (10). Taken together, these experiments indicate that the CNS parenchyma is able to profoundly modify the leukocyte responses to acute injury.

LPS is not directly chemotactic for leukocytes but it initiates myelomonocytic cell recruitment via resident tissue cells that release IL-1, TNF-α, and leukocyte chemotaxins like platelet-activating factor (PAF) and IL-8. The purpose of the present experiments was to determine whether the resistance of normal CNS parenchyma to recruitment of leukocytes could be overcome by direct injection of these known final mediators. We find that they induce microglial activation and leukocyte margination but not emigration into CNS parenchyma.

Materials and Methods

Reagents. FMLP and PAF were obtained from Sigma Chemical Co. (Dorset, England). Human r/IL-1α and β were obtained from Genzyme (Surrey, England). Murine r/TNF-α was a gift of Dr. S. Gillis (Immunex Corp., San Francisco, CA). Human r/IL-8 was a gift of Dr. I. Lindley (Sandoz Forschunginstitut, Vienna, Austria). Agents were LPS free by Limulus lysate assay.

Animals and Surgery. Adult BALB/c mice of either sex were obtained from the Sir William Dunn School (Oxford University), and C3H/HeJ mice were obtained from Harlan-Olac (Bicester, England). Stereotactic injections of 1 μl were delivered into the dorsal hippocampus through a pulled glass micropipette of total external diameter 0.15–0.2 mm, as described previously (9, 10). 1 μl was also injected into the skin of the ear using the same injection can-

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nula. Proinflammatory agents were dissolved in PBS or PBS with a 1% solution of monastral blue (Sigma Chemical Co.), which served as an inert tracer to locate the injection site and confirm delivery of the agent. Control injections were of 1% monastral blue-PBS or PBS alone.

Tissue Processing and Staining. Animals were deeply anesthetized and perfused with paraformaldehyde-lysine-periodate fixative after saline perfusion 4 h, 12 h, 1 d, 2 d, 5 d, and 7 d after injection as described (9, 10). 10-μm frozen sections and indirect immunohistochemistry with a polyclonal rabbit F4/80 antiserum were performed as described, and sections counterstained with cresyl violet to reveal nuclear morphology (9, 10). The presence of neutrophils was detected by their characteristic multilobed nuclei, and macrophages and microglia by F4/80 immunocytochemistry. F4/80 is a plasma membrane antigen of unknown function that is specific for macrophages and microglia in the CNS (11, 12), and no staining was detected in the absence of first antibody. Independent experiments were performed with at least two animals per time point.

Results

IL-1 and TNF. The intrahippocampal injection of 0.5 U IL-1α with 1 U IL-1β produced no leukocyte recruitment to the CNS parenchyma despite the substantial IL-1 receptor distribution on neurons (13) (data not shown). We therefore injected large doses (up to 50 U IL-1α with 100 U IL-1β or 10^4 U TNF-α). To our surprise there was trivial neutrophil exudation into the CNS parenchyma: rather than any myelomonocytic infiltrate as observed when injections were made into the skin (Fig. 1 *a*), only occasional isolated neutrophils were found (Fig. 1, *d–g*). At the high doses, the paucity of recruitment was the more conspicuous for the striking margination of neutrophils to blood vessels over a wide area up to a few millimeters from the injection site, indicating that the cytokines had diffused within the parenchyma (Fig. 1 *g*). Margination was evident from 4 to 24 h and maximal at 12 h after injection. The amount of margination observed for IL-1 was much greater than that after TNF, and most likely reflects the larger proinflammatory dose of IL-1 used. There was also no significant recruitment of monocytes into the parenchyma after IL-1 or TNF injection. Apart from the limited reaction in the cannula track similar to that with control vehicle injections and described previously (10), the normal regular distribution of F4/80$^+$ cells (microglia) in the neurophil was unchanged by the cytokine injections (Fig. 1, *d* and *e*) and there was no increase in their number (data not shown). However, instead of normal uniform F4/80 staining intensity (14), microglia in the injected hippocampus and overlying cortex displayed increased staining intensity from 12 to 24 h compared with microglia elsewhere in the section. Some microglia within a few hundred microns of the injection site displayed shortened cell processes and increased arborization over the first 24–48 h of the time course (Fig. 1 *f*). Thus, IL-1 and TNF-α had effects on both microglia (morphologic activation) and on cerebral endothelium (neutrophil margination), but minimal leukocyte recruiting action.

While there was minimal leukocyte recruitment into CNS parenchyma, the intrahippocampal injections of IL-1 or TNF-α, even at low doses, induced a typical acute myelomonocytic inflammatory response in the ventricular system that is anatomically distant from the site of delivery and beyond the region of parenchymal microglial activation or leukocyte margination. This distant activity presumably resulted from diffusion through extracellular CSF to the choroid plexus. Numerous leukocytes were present in the ventricles and subarachnoid space from 4 to 24 h, and also in choroid plexus stroma at 4 h (Fig. 1, *b* and *c*). By 12 h the choroid plexus was normal, but leukocytes were still present in the ventricles and subarachnoid space, especially in recesses of the third ventricle ipsilateral to the injected hemisphere. By 48 h leukocytes were no longer present in the ventricles. In control experiments, there were no leukocytes in the subarachnoid space, ventricles, or choroid plexus when vehicle (PBS or PBS/monastral blue) was injected alone, and the tracer had no effect on the responses to cytokine injections. Similar leukocyte responses were also found in cytokine-injected C3H/HeJ mice, indicating that they were not caused by LPS contamination.

FMLP, PAF, and IL-8. IL-1 and TNF are not directly chemotactic for leukocytes in vitro (15) and their leukocyte recruiting effects are mediated by resident tissue cells. The next series of experiments tested whether a potent leukocyte chemotaxin placed in the CNS parenchyma would be able to induce leukocyte emigration into the tissue, as predicted by its actions in vitro.

Subcutaneous injections into the ear of FMLP (10–1,000 pmol), PAF (1–10 nmol), or IL-8 (10–1000 ng) with monastral blue tracer induced extensive neutrophil infiltration at 4 h (data not shown). After intrahippocampal injection, there was a neutrophil inflammatory response in the ventricular system, choroid plexus, and meninges, as seen for IL-1 and TNF (Fig. 2 *a*). However, at the actual injection site in hippocampus there were very few neutrophils and not substantially more than the response to vehicle alone, despite the delivery of large doses of agent (Fig. 2, *b–e*). The intracerebral injection of the largest dose of IL-8 tested (1 μg) was exceptional in that it produced neutrophil exudation into the parenchyma but it was within the vicinity of the injection track, although dense neutrophil margination was observed in vessels up to several millimiters away (Fig. 2, *b* and *c*). All the chemotaxins induced neutrophil margination and morphologic microglial activation in the injected hippocampus and overlying cerebral cortex from 12 h after injection; F4/80 staining intensity increased compared with that present in other regions of the tissue section, and many cells in this region displayed shortened processes for the next 2 d. Thus, a range of chemotaxins having diverse chemical structures similarly failed to induce significant leukocyte recruitment to the CNS parenchyma.

Discussion

Four striking findings were made in the present experiments. First, the single bolus delivery of proinflammatory cytokine IL-1 or TNF, or the leukocyte chemotaxin FMLP, PAF, or IL-8 into the normal adult hippocampus, failed to induce significant myelomonocytic recruitment to the paren-

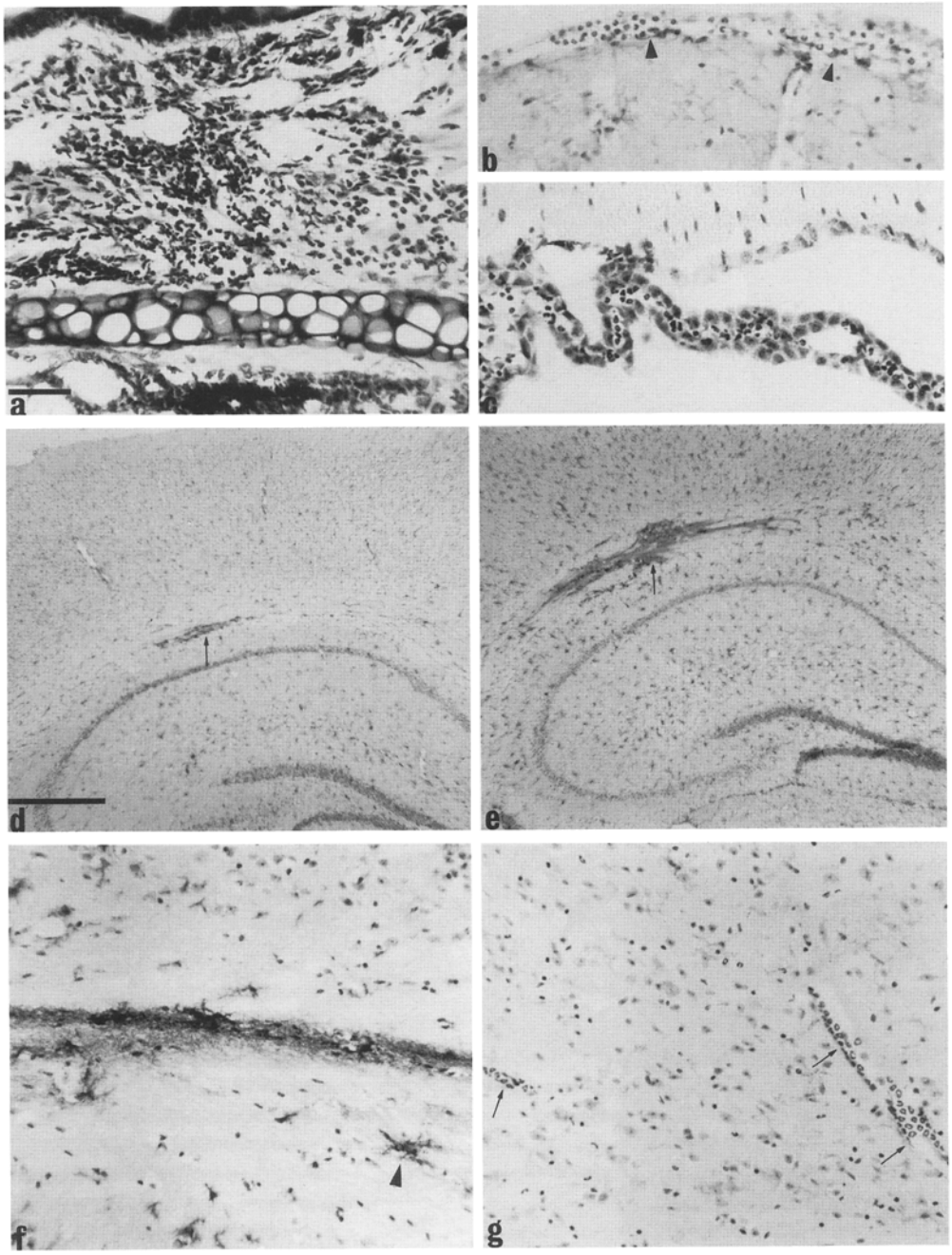

Figure 1. Cresyl violet– and F4/80-stained sections to show leukocyte responses to intrahippocampal injection of IL-1 or TNF-α at 12 h. Subcutaneous injection of 50 U IL-1α with 100 U IL-1β in the ear induces a large myelomonocytic infiltrate on both sides of the cartilage at this time (*a*). When injected into the hippocampus there is a myelomonocytic infiltrate in the subarachnoid space (*arrowheads*) (*b*), and choroid plexus (at 4 h) (*c*), but not at the site of injection (*d*) (tracer indicated by *arrow*). High-power views of the parenchymal response show that IL-1-induced morphological activation of microglia (*arrowhead*) (*f*), and leukocyte margination (*arrows*) (*g*) but minimal diapedesis. Injections of 10^4 U TNFα induced similar leukocyte and microglial reactions (*e*) (bars, 50 μm: *a*,*b*,*c*,*f*, and *g*; and 0.5 mm: *d* and *e*).

chyma as they do in other tissues. Of note in this regard, chronic TNF release by intracerebral TNF-secreting tumors also does not induce inflammation in the CNS parenchyma (16). Thus, the highly modified leukocyte responses in CNS parenchyma during neuronal degeneration or after LPS injection (9, 10) are also observed with several different mediators of leukocyte recruitment, and therefore even if they are produced in these lesions, would be expected to have minimal acute recruiting activity.

Second, since leukocyte chemotaxins failed to induce recruitment into parenchyma, it indicates that a chemotaxin in vitro is not necessarily one in vivo and that their leukocyte recruiting ability is not mediated solely through the creation of a chemotactic gradient across a blood vessel. Evidence for this already exists in nonneural tissues (the tachyphylaxis phenomenon) in which repeated intradermal injection of a chemotaxin induces local desensitization of leukocyte recruitment to that specific agent (17).

Third, margination could be separated from diapedesis in CNS parenchyma indicating that they are distinct events and that inhibition of leukocyte recruitment lies at the latter step. It must be remembered that margination and microglial ac-

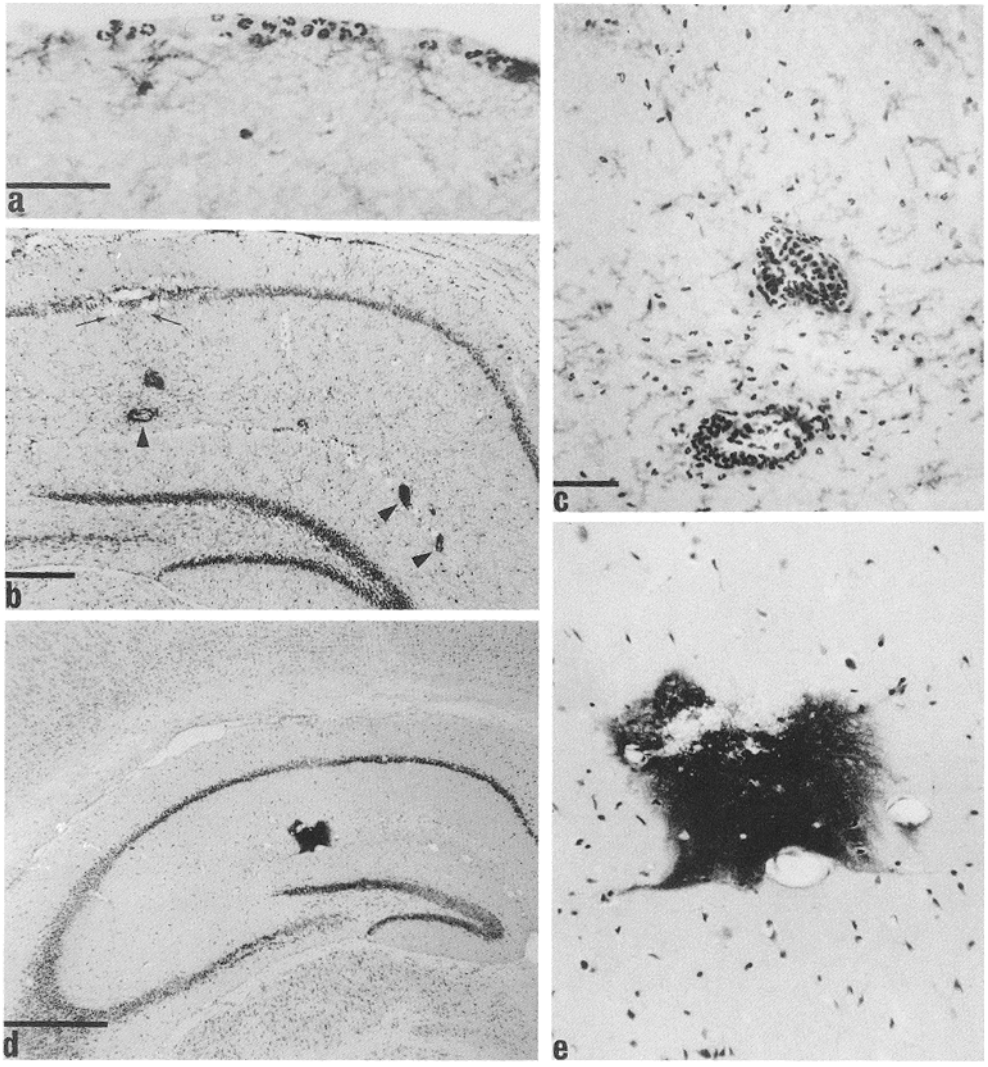

Figure 2. Different leukocyte responses at 12 h after intrahippocampal injection of chemotaxins. The injection of 1 μg IL-8 induces abundant neutrophil infiltration of the subarachnoid space (*a*), but in parenchyma it is confined to the region of the cannula track (*arrows*) and is minor (*b* and *c*) though there is dense margination visible in the large vessels at this magnification (*arrowheads*) (*b*). The injection of 100 pmol FMLP with tracer also fails to induce a myelomonocytic infiltrate in the parenchyma (*d* and *e*) (bars, 50 μm: *a*,*b*,*c*, and *e*; and 0.5 mm: *d*).

tivation were only readily observed with large doses; more physiological ones had negligible effect in the parenchyma.

Finally, the intrahippocampal injection of all the proinflammatory mediators induced rapid myelomonocytic cell recruitment to the choroid plexus and ventricular system, as they do when injected into other tissues. These findings are in agreement with those of previous workers who showed IL-1 and TNF delivered intracisternally induced rapid myelomonocytic recruitment to the CSF (5, 6). However, our findings here and previously (10) show that leukocyte inflammatory responses in the choroid plexus–CSF compartment are independent of those in the CNS parenchyma and from which they must be distinguished. We do not know whether the inhibition of leukocyte recruitment is due to the cerebral endothelium, which has a different morphologic structure (18), antigen phenotype (19, 20), and response to cytokine stimulation in vitro (21) compared with endothelium elsewhere; to the microglia, which are immunologically downregulated compared with macrophages in the choroid plexus, ventricles, meninges, or elsewhere in the body (22); or to a cell-associated or extracellular matrix component of parenchyma that inhibits leukocyte entry. The precise mechanisms are of considerable interest not only in understanding the pathogenesis of CNS disease, but also for possible antiinflammatory application in other tissues.

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