Brain-blood permeability: TNF-α promotes escape of protein tracer from CSF to blood

JODI B. DICKSTEIN,1 HARVEY MOLDOFSKY,1 AND JOHN B. HAY2
1Centre for Sleep and Chronobiology, 2Departments of Immunology and Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada M5T 2S8

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Dickstein, Jodi B., Harvey Moldofsky, and John B. Hay. Brain-blood permeability: TNF-α promotes escape of protein tracer from CSF to blood. Am J Physiol Regulatory Integrative Comp Physiol 279: R148–R151, 2000.—The objective of this study was to determine the effect of tumor necrosis factor (TNF)-α on the efflux of protein from the central nervous system to blood based on assessing the clearance of radiolabeled albumin from the cerebrospinal fluid (CSF) to blood in rats. 125I-labeled human serum albumin (125I-HSA) was injected into a lateral ventricle, and venous blood was sampled hourly to determine the basal CSF protein clearance into the blood. After this, rats were intraventricularly infused with 10 μl TNF-α and 10 μl 131I-HSA (n = 6) or 10 μl saline and 10 μl 131I-HSA (n = 6). Venous blood was sampled hourly for 3 h. 131I-HSA tracer recovery increased threefold in the venous blood and was significantly higher in the spleen, muscles, and skin in animals treated with TNF-α. No significant changes were observed in control animals treated with saline. The data suggest that TNF-α promotes the clearance of protein macromolecules from the CSF to the venous blood.

MATERIALS AND METHODS

Animals. Experiments were performed on 12 male Wistar rats (Charles River Breeding Laboratories, Quebec, Canada) weighing between 225 and 400 g. Animals were housed in individual cages and maintained on a 12:12-h light-dark cycle (0600–1800 light) with food and water supplied ad libitum.

Tracers and solutions. Rat TNF-α was obtained from R & D (Minneapolis, MN) and reconstituted in sterile saline. 125I-labeled human serum albumin (125I-HSA) and 131I-labeled human serum albumin (131I-HSA) were obtained from Draximage (Quebec, Canada).

Surgeries. All surgeries were performed under sterile conditions. Anesthesia was initiated with a mixture of ketamine HCl and acepromazine (1:1) intraperitoneally and maintained by supplemental doses as required. An incision was made in the rat’s scalp to expose the coronal sutures. A 22-gauge needle was used to burr a hole 2 mm caudal to the coronal suture and 2 mm lateral to the sagittal suture. A guide cannula (Plastics One, Roanoke, VA) was introduced into one of the lateral ventricles and secured to the skull with cyanoacrylate glue and dental acrylic cement. At the end of the experiment, Evans blue dye was injected into the lateral ventricle to confirm the placement of the cannula.

After a 1-wk recovery period, a polyethylene tube (0.58 mm ID 0.96 mm OD) was implanted into a jugular vein and passed subcutaneously through a small incision in the nape of the neck. This permitted the sequential sampling of venous blood. Patency of the catheter was maintained using a heparinized saline flush.

Protocol. To determine the effects of TNF-α on CSF albumin clearance into the blood, we employed a two-stage protocol. 125I-HSA (10 μl) was introduced into a lateral ventricle in each of the 12 anesthetized rats. The recovery of tracer was monitored in the blood hourly for 3 h. After 3 h, the rats were divided into two equal groups (n = 6). Saline (10 μl) and 131I-HSA (10 μl) were introduced into the lateral ventricle of the control group of rats, while 10 μl TNF-α (250 ng) and 10 μl 131I-HSA were introduced into the lateral ventricle of the experimental group of rats. All injections were performed at 0900 h.

Address for reprint requests and other correspondence: J. B. Dickstein, CMCC, 1900 Bayview Ave., Toronto ON, M4G 3E6, Canada (Email: jodi.dickstein@utoronto.ca).

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a rate of 1 μl/min. Venous blood was sampled hourly for the next 3 h. At the end of the experiment, rats were killed (Euthanyl, euthanasia solution, MTC Pharmaceuticals, Cambridge, Ontario)and lymph nodes and tissues were excised and weighed. 125I- and 131I-HSA were measured in 100-μl aliquots of blood plasma, and 131I-HSA was measured in tissue and lymph nodes using an LKB 1282 Compu-Gamma CS LKB Wallace (Pharmacia, Turku, Finland). Previous studies have demonstrated that 125I and 131I detected in serum is attached to albumin 6 h postinjection (3–5, 11).

Tracer recovery in tissue was analyzed using Student’s ANOVA and Student-Newman-Keuls multiple comparison. Values are expressed as a percent of the initial injected dose/g tissue ± SD. Plasma recovery data were analyzed using ANOVA and Student-Newman-Keuls multiple comparison. Tracer recovery in tissue was analyzed using Student’s t-test. P values of <0.05 were considered significant.

RESULTS

HSA recovery in plasma. Plasma recoveries of radio-labeled HSA from control and TNF-α-treated animals are illustrated in Fig. 1. In stage 1, 125I-HSA was infused into the lateral ventricle of all animals and plasma tracer concentration was measured. In stage 2, 131I-HSA was infused into the lateral ventricle in conjunction with TNF-α or the saline control. The recovery of 131I-HSA tracer in the plasma of TNF-α-treated animals increased threefold by injection hour 3 (P < 0.05) compared with the basal CSF protein clearance as determined by 125I-HSA. There were no significant differences in the recovery of plasma 125I- and 131I-HSA in saline-treated rats.

HSA recovery in the nodes and tissues. 131I-HSA was measured in lymph nodes and in tissues in control and TNF-α-treated rats (Table 1). There were no significant differences in 131I-HSA recovery in lymph nodes of animals treated with TNF-α or saline. The recovery of 131I-HSA in the spleen, muscle, and skin in TNF-α-treated animals was significantly greater than saline-treated animals.

DISCUSSION

This study demonstrates that TNF-α increased the efflux of protein from the brain into the blood as measured by radioiodinated serum albumin. The concentrations of 131I-HSA in the blood plasma and spleen were significantly greater in rats treated with TNF-α compared with rats treated with the saline control. The results suggest that TNF-α promotes the clearance of protein macromolecules from the CSF to the venous blood.

The arachnoid villi provide one route for CSF fluid regulation. The action of TNF-α on the arachnoid membrane to promote increased protein clearance remains unclear. TNF-α is known to induce morphological changes in endothelial cells. TNF-α increases the permeability of the blood-brain barrier (10, 16) and of endothelial cell monolayers (7, 17) as early as 1–3 h postexposure. TNF-α induces G protein-mediated conformational changes in the actin-based cytoskeleton that occur concomitant with cell retraction resulting in intercellular gaps (7). A disrupted arachnoid membrane may account for the increased tracer recovery in the blood compared with the saline-treated animals.

Increased levels of 131I-HSA in TNF-α-treated animals may be caused by an increase in intracranial pressure. CSF regulation at the level of the arachnoid villi is dependent on pressure differences between the CSF and dural venous sinus (20). Tureen (19) demonstrated...
strated that intracisternal injection of TNF-α increased intracranial pressure in rabbits. This increased intracranial pressure was associated with increased cerebral blood flow, mediated through the activity of nitric oxide. However, Angstwurm et al. (1) were unable to confirm these findings in the rat. In addition, intracerebroventricular TNF-α evokes an inflammatory response accompanied by an influx of leukocytes into the CSF (14). TNF-α administered into the CSF increases permeability to sodium fluorescein (16) and albumin (14). The influx of macromolecules into the brain and CSF due to TNF-α enhanced blood-brain-barrier permeability has the potential to increase intracranial pressure. It is therefore conceivable that the increased CSF protein clearance to the blood following the administration of TNF-α was due to an increase in intracranial pressure.

TNF-α is associated with both vasodilator (18) and vasoconstrictor (15) properties on blood vessels. Our study did not address the effect of TNF-α on blood vessels. It is unlikely that the elevation of protein tracer in TNF-α-treated animals was a result of TNF-α-induced vasoconstriction. Vasoconstriction alone cannot account for the threefold difference in albumin recovery observed in the TNF-α-treated animals, because the blood volume could not be reduced by such a magnitude.

Intraventricular TNF-α enters the blood with the reabsorption of CSF (8, 12). The increased HSA recovery in TNF-α-treated animals may be a result of TNF-α entering the circulation via the sagittal sinus and acting at a peripheral site. Dose-response studies indicate similar potencies for TNF-α following central or peripheral administration in inducing anorexia in rats (2). In contrast, intravenously administered TNF-α failed to induce centrally mediated TNF-α changes in blood-brain-barrier permeability (14). Furthermore, cytokines entering the blood with the reabsorption of CSF become diluted in the entire blood volume. On the basis of existing literature, the increased efflux of HSA may be centrally and/or peripherally mediated. A subsequent series of experiments should be directed at this possible mechanism.

The elevated recovery of tracer in the blood in TNF-α-treated animals is not attributed to increased intracranial pressure due to volume loading. HSA and TNF-α solutions were microinfused at a slow rate of 1 ml/min, which is well below the rate of CSF formation in the rat (9). This slow infusion would have prevented any sudden elevation of CSF pressure. Furthermore, if this were true, tracer recovery in saline-treated rats would have been elevated in the plasma, which was not the case.

In addition to the arachnoid villi route, CSF drains along perineural extensions of the subarachnoid space directly into the regional cervical lymphatics (13). Boulton et al. (3) demonstrated that incremental changes in intracranial pressure were associated with higher CSF transport through both the lymphatic and the arachnoid villi routes. We examined the possibility that TNF-α could increase the transport of labeled albumin out of the cranial vault into the extracranial lymphatics by determining the radioactivity in the nodes 3 h after the injection of TNF-α or saline. Lymphatics were not cannulated because of the technical difficulties related to the small size of lymphatic vessels in the rat. Our results show that tracer was increased in the spleen, muscles, and skin, but not in the lymph nodes. In a previous study, we showed that both radiolabeled albumin and TNF-α injected into the lateral ventricle of a sheep could be recovered in efferent cervical lymph and venous blood (11). The total recovery of radiolabeled TNF-α and albumin tracer in the blood was much greater than that recovered in the lymph. These data, along with the present study, suggest that TNF-α may preferentially enhance CSF clearance into the blood and not the lymphatics.

In summary, this study demonstrates that TNF-α increases the efflux of protein from the CSF into the blood. This finding may play an important role in disease states such as multiple sclerosis, meningitis, and cerebral edema, in which TNF-α levels are elevated in the brain and CSF.

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REFERENCES


