

## Passage of Immunomodulators Across the Blood-Brain Barrier

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The question is considered of how and where cytokines, such as interleukin 1 (IL-1), that are released into the circulation during the host defense response, reach and interact with the central nervous system to produce fever or act as neuroimmunomodulators. Evidence is presented suggesting a role for a brain circumventricular organ (CVO) in this respect. Several interactions between a specific CVO, the organum vasculosum laminae terminalis (OVLT) and endogenous pyrogen (EP) in the production of fever are reviewed. A more general hypothesis is developed on a role for the brain CVOs in monitoring the blood concentrations of several proteins and complex polypeptides such as the circulating endocrines that are regulated via the autonomic nervous system. A proposed connection between the release of prostaglandin E (PGE) at the blood-brain interface in response to infection and the ability of the brain to maintain an immunoprivileged status in the face of exposure of its CVOs to foreign antigens is discussed.

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### THE BLOOD-BRAIN BARRIER AND THE PATHOGENESIS OF FEVER

During the host defense response to infection or injury, a variety of bone marrow-derived cells release into the circulation several cytokines, such as interleukins (IL-1 and IL-6) and tumor necrosis factor (TNF), that are collectively known as endogenous pyrogen (EP). An important problem in any discussion of the pathogenesis of fever is whether or not EP enters the brain neuropil from the circulation in order to produce the activation of thermogenesis and the inhibition of heat loss mechanisms that result in the characteristic increase in body temperature known as fever. Despite studies using labeled EP [1] which were unable to demonstrate the presence of EP within the brain during fever production, it is still widely believed that EP exerts its fever-inducing effects on the central nervous system (CNS) by entering and acting within the preoptic/anterior hypothalamic (PO/AH) region of the hypothalamus. It is thought that EP interacts within the brain neuropil to form the cyclo-oxygenase product prostaglandin E (PGE), which in turn acts upon the CNS thermoregulatory neurons to induce fever (see Fig. 1).

There are, however, several reasons to question this particular series of events in the pathogenesis of fever. First, studies that have examined the distribution of systemically injected labeled EP [1] or IL-1 [2] both in the brain and the body have shown that,

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*Abbreviations:* AP: area postrema AV3V: anteroventral region of the third ventricle CNS: central nervous system CVO: circumventricular organ EP: endogenous pyrogen HRP: horseradish peroxidase icv: intracerebroventricular(ly) IL-1: interleukin 1 iv: intravenous(ly) LPS: lipopolysaccharide(s) MDP: muramyl dipeptide OVLT: organum vasculosum laminae terminalis PGE: prostaglandin E PO/AH: preoptic/anterior hypothalamic (region) SFO: subfornical organ TNF: tumor necrosis factor

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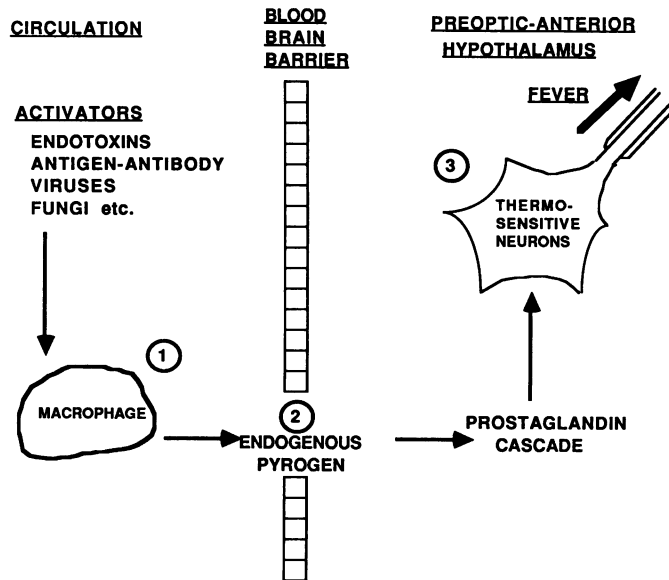


FIG. 1. An early hypothesis on the pathogenesis and mechanisms of the febrile event. A variety of pathogens (activators) stimulate bone marrow-derived cells to elaborate and release EP into the circulation (1). This polypeptide travels via the cerebral circulation to the blood-brain barrier, where it is believed to enter somehow the PO/AH neuropil (2). The EP is then thought to stimulate the production of prostaglandins from an unknown cell type within the brain. The prostaglandins such as PGE are then believed to alter the thermosensitivity of temperature-sensitive neurons in the PO/AH area in such a manner as to cause fever (3).

while these labeled cytokines can be readily detected in most peripheral tissues, they do not appear to cross the blood-brain barrier and enter the brain parenchyma. Second, there are fundamental differences in the dynamics and timing of fevers that are produced when EP or IL-1 are injected directly into the brain cerebroventricles compared to when they are injected intravenously. Surprisingly, the latency to fever onset and the duration of fevers induced by the intravenous route are shorter and more clear-cut than when they are induced by the intracerebroventricular route [3]. This finding led us to postulate that the site of action of EP is closer to the circulation than the brain neuropil and to consider specialized areas of the cerebral vasculature as possible sites of entry or action of EP in the production of fever. Particularly noteworthy in this regard are the seven or eight so-called circumventricular organs (CVOs), located at various sites within the walls of the cerebral ventricles of the brain. These highly vascularized regions are distinct from the brain parenchyma and their capillaries differ in their permeability properties from the tight-junctioned, non-fenestrated capillaries that are universally found in the rest of the cerebral circulation.

Weindl et al. have published several studies of the relationship between the brain CVOs and the cerebral circulation on one side, and the brain neuropil on the other. Using the reaction product of horseradish peroxidase (HRP) as a marker, they have mapped the distribution of this 45,000 dalton protein within the CVOs and the brain after it had been injected into the circulation or into the cerebroventricular space

[4,5,6,7]. Their findings showed that when HRP was injected intravenously (iv), it did not enter the brain parenchyma, due to the blood-brain barrier. It did appear, however, to be contained within the interstitial spaces of the CVOs that are found abutting the cerebroventricular system of the brain. On the other hand, when HRP was injected intracerebroventricularly (icv), its reaction product distributed uniformly throughout the entire brain parenchyma but was excluded from the interstitia of the CVOs.

This finding led Weindl to conclude that, while the interstitial spaces of these CVOs were in direct contact with the cerebral circulation, they did not permit the passage of the normally vascular-permeable HRP proteins into the brain neuropil. The capillary endothelial cells within the CVOs were fenestrated and thus permitted the entry of HRP into the CVO; however, the impermeability of the brain parenchyma to HRP from the circulation side of the CVOs was due to the presence of tight-junctioned ependymal cells surrounding the border between the CVO and the brain neuropil. These same tight-junctioned ependymal cells also appeared to act as a barrier to the movement of HRP from the brain parenchyma into the CVO, when HRP was injected into the cerebral ventricles. He further postulated that the CVOs might have a role in either secreting substances into the cerebral circulation or in monitoring the concentration of complex proteins in the cerebral circulation in connection with the brain's role of controlling various autonomic endocrine functions [6]. Weindl also described in some detail the vascular anatomy of several of these CVOs [7,8].

#### THE OVLT AND THE PATHOGENESIS OF FEVER

These observations led us to consider a possible role for one particular CVO, the organum vasculosum laminae terminalis (OVLT), in connection with the pathogenesis of fever. The OVLT was chosen because of its proximity to the PO/AH area. Our initial study consisted of placing discrete electrolytic lesions within the confines of the OVLT in rabbits and rats in order to ascertain whether these had any effect on the development of fever in response to intravenously injected EP [9]. Much to our surprise, we found that these small lesions greatly augmented the febrile response to EP and that the enhanced febrile responses, which were maximal at between three to six days after the lesioning, gradually diminished over a period of three to four weeks (Fig. 2). Appropriate control lesions posited within the PO/AH region had little effect on fever production. Blatteis and his colleagues [10] also showed that more extensive lesions encompassing the entire anteroventral wall of the third ventricle (in which OVLT is located), abolished the febrile responses of guinea pigs that were subsequently given intravenous injections of endotoxin pyrogen. They interpreted their results as showing that pyrogen gained entry into the PO/AH area via the anteroventral region of the third ventricle (AV3V) and that lesioning subsequently prevented the entry of the pyrogen into the brain. It is equally likely, however, that these much larger AV3V lesions ablated the entire OVLT and, with it, the receptor sites to EP that produce PGE (*vide infra*).

Further research of the literature in this area revealed two histological studies on the composition of the OVLT by Sano, describing the existence of a mesenchymal cell type, within the interstitium of the OVLT, which had phagocytic properties similar to reticuloendothelial cells and which endocytosed intravenously injected HRP [11,12]. We had also previously observed enhanced febrile responses to EP (iv) in rats several days after they had been injected intravenously with endotoxin (a lipopolysaccharide which is known to stimulate phagocytosis in the reticuloendothelial system). Therefore,

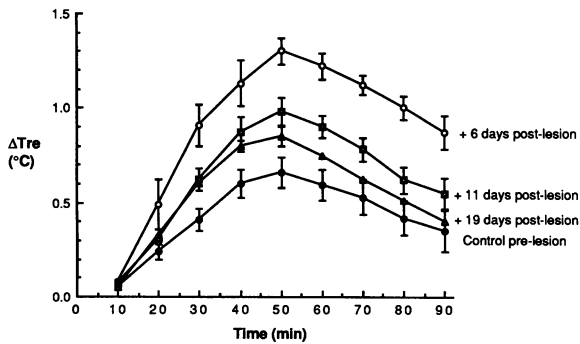


FIG. 2. Enhancement of the febrile response of rabbits to a standard 0.5 ml/kg dose of EP (iv) at 6, 11, and 19 days after small lesions were placed in the OVLT, compared with the pre-lesion control febrile response to the same dose of EP.  $\Delta T_{re}$  = mean increase in rectal temperature  $\pm$  SEM (all three curves are significantly different from control curve).

we wondered whether the small lesions that we had made in the OVLT region might have stimulated phagocytosis in these mesenchymal cells in the OVLT, which in turn might account for the enhancement of the febrile response to EP that we had observed after the lesioning. Thus, we decided to examine the effect of injecting zymosan into the circulation on EP fever production in rats, since zymosan, in common with other immunoadjuvants such as lipopolysaccharides (LPS) and muramyl dipeptide (MDP), is known to stimulate the phagocytic activity of reticuloendothelial cells. We found that three days after rats were injected with zymosan (30 mg/kg, iv), their febrile response to an intravenous injection of EP or IL-1 was markedly enhanced. This febrile enhancement lasted for a period of several weeks (see Fig. 3), gradually declining, in a manner similar to the fever enhancement we had observed after lesions were placed in the OVLT. Similar enhancements of EP fever were obtained when rats were pre-treated with either LPS or MDP, but not when we injected sterile latex beads [13].

To determine whether these immunoadjuvants might be acting at the OVLT region to produce this enhancement, we implanted rats with microinjection cannulae over the OVLT region of the brain and tested their febrile responses to EP both before and after

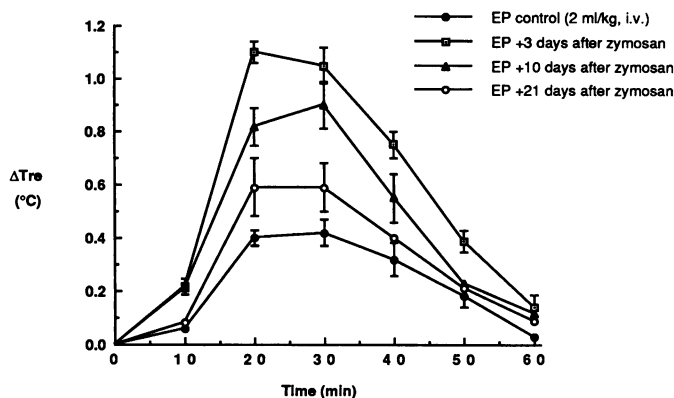


FIG. 3. A study of the duration of the fever-enhancing effect of zymosan pre-treatment in rats (30 mg/kg iv) in response to a standard dose of EP (2.0 ml/kg iv). Maximum fever enhancement occurred between days 3 and 10 after zymosan treatment and, thereafter, the febrile response declined toward the febrile control level.  $\Delta T_{re}$  = mean increase in rectal temperature  $\pm$  SEM (all three curves are significantly different from control curve).

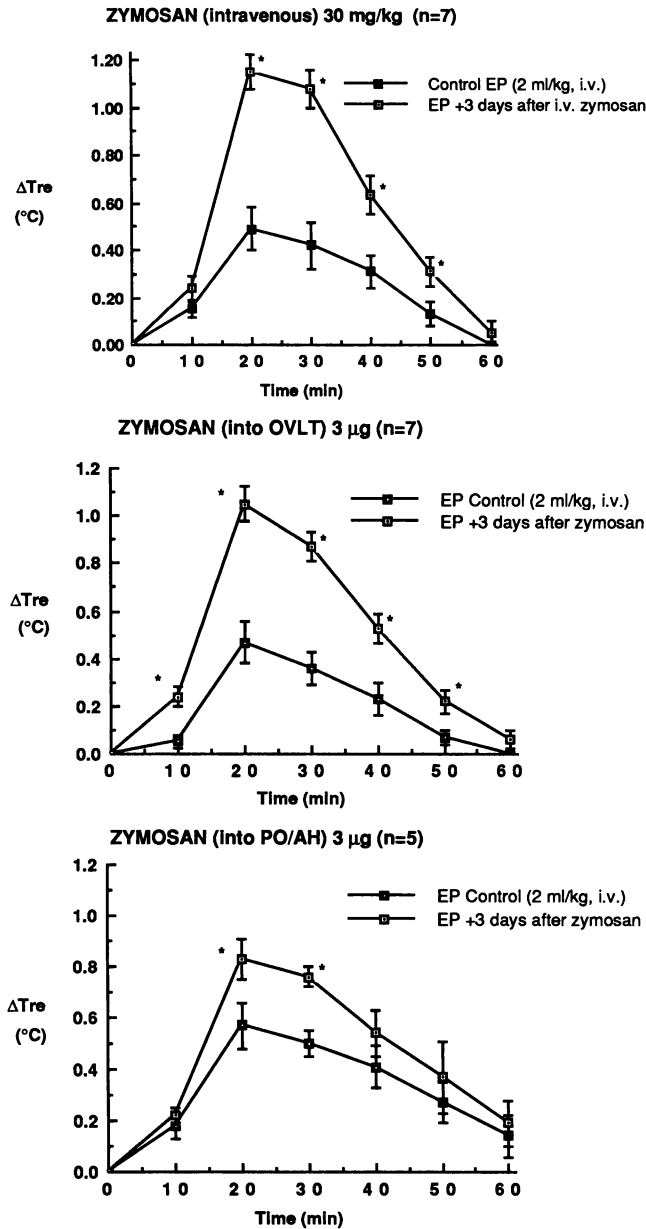


FIG. 4. A comparison of the fever-enhancing effects of zymosan in rats when it is delivered. **A.** intravenously (30 mg/kg); **B.** into the OVLT (3  $\mu$ g); **C.** into the PO/AH (3  $\mu$ g). Note that the fever enhancement produced by intra-OVLT injection of zymosan emulates that obtained after intravenous injection, whereas intra-PO/AH injection produces minimal fever enhancement.  $\Delta T_{re}$  = mean increase in rectal temperature  $\pm$  SEM; \* indicates  $p > 0.05$ .

the introduction of smaller (3  $\mu$ g) quantities of zymosan directly into the OVLT region. This treatment produced enhanced febrile responses to EP, similar to those observed after the iv injection of much larger amounts of zymosan. Injection of identical small amounts of zymosan into the adjacent PO/AH area or into the cerebroventricular system had little or no effect on subsequent EP fevers [14]. A summary of the results of these studies is shown in Fig. 4. Similar fever enhancements were also produced by the introduction of tiny amounts of LPS and MDP into the OVLT region of the rats.

Therefore, we concluded that the immunoadjuvants, which when injected intrave-

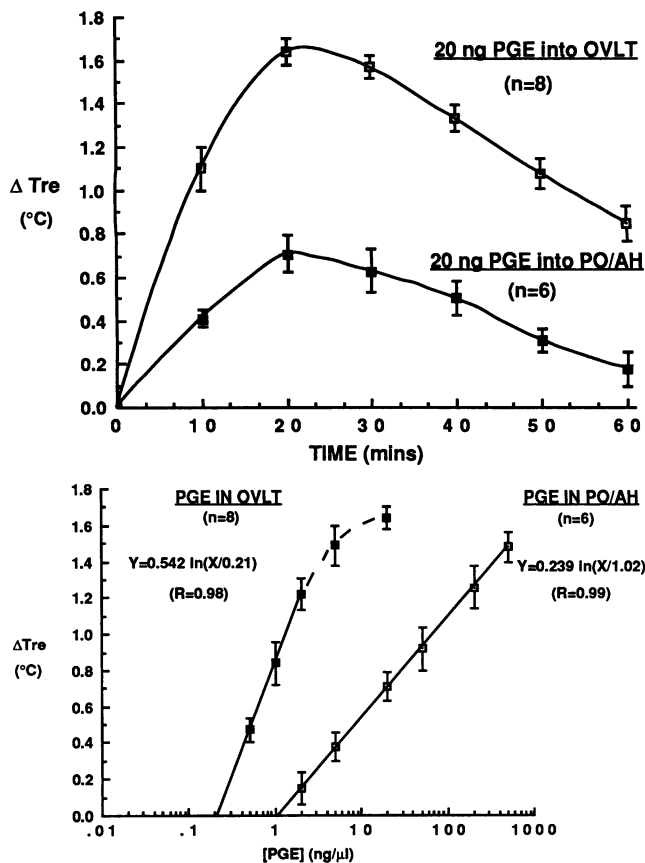


FIG. 5. A. A comparison of the changes in rectal temperature ( $\Delta T_{re}$ ) in two groups of rats, measured at ten-minute intervals after the microinjection of 20 ng of PGE into the OVLT region and the PO/AH area. B. A comparison of the febrile dose responses to PGE, microinjected into the OVLT and the PO/AH in these two groups of rats.  $\Delta T_{re}$  = mean increase in rectal temperature  $\pm$  SEM.

nously had enhanced EP fevers in rats, were acting at or near the OVLT region and were making the rats more sensitive to EP. One possible explanation of this enhanced febrile response to EP in rats after immunoadjuvant treatment was that the release of PGE in response to EP was augmented after immunoadjuvant treatment. We wondered if the OVLT might be the actual site of PGE release in response to intravenously injected EP. It is generally reported that prompt and high fevers are induced when nanogram quantities of PGE are microinjected directly into the PO/AH region of the brain of nearly all mammalian species [15]. A first step to testing this hypothesis was to inject PGE into the OVLT rather than into the PO/AH and ascertain if it was capable of producing fever in rats. The results are illustrated in Fig. 5.

Not only did PGE produce fevers when it was injected into the OVLT region, but the fever sensitivity of this area of the brain to PGE far exceeded that of the adjacent PO/AH region [16]. This result appears to support the hypothesis that the OVLT is not only the site of interaction for EP between the circulation and the brain, but that it is also the site at which EP causes the release of PGE, which in turn is thought to produce the febrile response by acting on the neurons in the PO/AH area that regulate body temperature. The greater sensitivity of the OVLT region to PGE in the production of fever in rats suggests that those neurons that are the presumed targets of PGE must also lie very close to the OVLT, although the relatively small, lipophilic PGE molecule would have little trouble in penetrating the blood-brain barrier at any

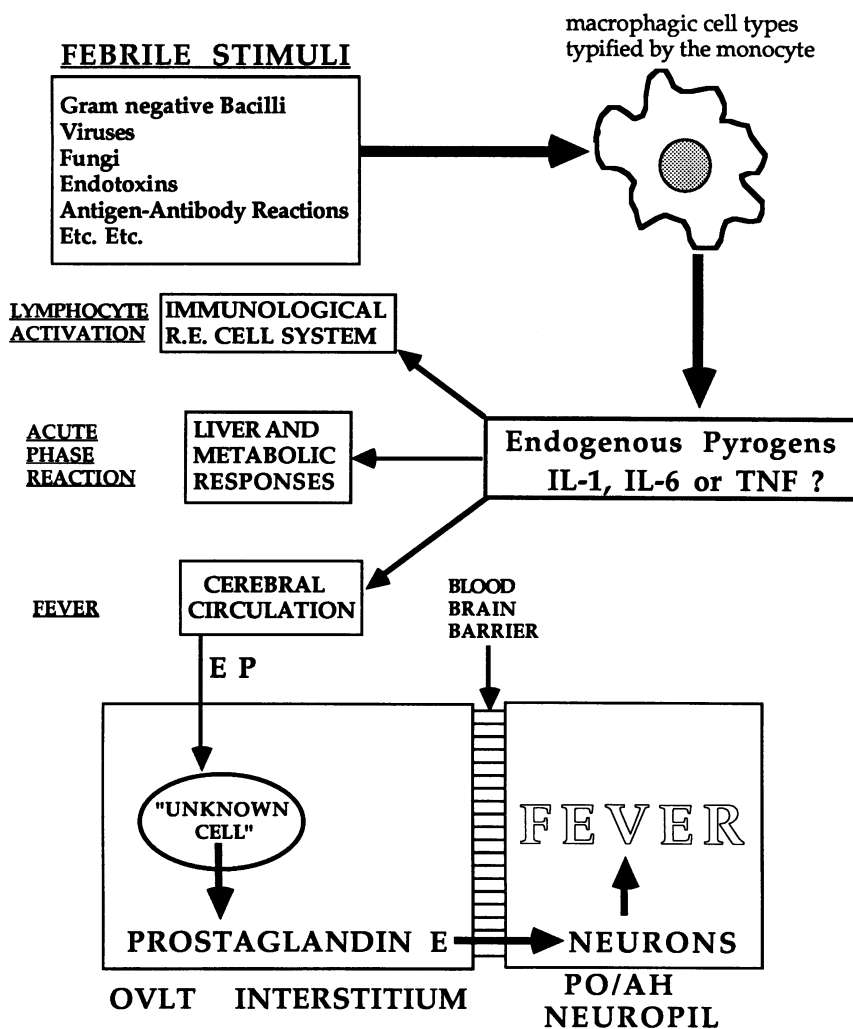


FIG. 6. A modified hypothesis encompassing the action of cytokines such as IL-1, IL-6, and TNF in the pathogenesis of immune responses, the acute-phase response, and fever. A variety of pathogens (activators) stimulate bone marrow-derived cells (here represented by a macrophage) to elaborate and release these cytokines, collectively termed EP, into the circulation. The mitogenic immune responses and the acute-phase response appear to occur via the systemic action of one or more of these cytokines. EP also travels via the cerebral circulation to the OVLT, where it is believed to stimulate the production of prostaglandin E from an unidentified cell type. PGE is then thought to enter the adjacent brain neuropil and to cause fever by altering the activity of temperature-sensitive neurons in the PO/AH area.

location in the cerebral circulation. It may be that the cells described by Murabe et al. [12] are those which produce PGE in response to circulating EP in the pathogenesis of fever, although this suggestion awaits direct investigation. Nevertheless, Fig. 6 illustrates a modified hypothesis on the host defense response and the pathogenesis of fever, incorporating these recent findings and our current speculations.

While a putative role for a CVO such as the OVLT in the pathogenesis of fever is intriguing, a larger question of the role of CVOs in general might also be considered.

For example, is the release of PGE at the OVLT, in response to the cytokines that comprise EP, solely a link in the pathogenesis of fever? Or is it just one manifestation of a larger role for PGE and the CVOs in the normal interaction and communication between the brain and the circulation? Does the release of PGE within the OVLT in response to circulating EP have any other effect at the blood-brain interface, beyond the production of fever?

#### A SENSORY ROLE FOR SOME CIRCUMVENTRICULAR ORGANS?

It is widely believed that a variety of proteins and complex polypeptides concerned with autonomic function are monitored by central nervous system structures such as the hypothalamus and septal area [17]. For example, the majority of the circulating hormones such as thyroxine, corticosteroids, and gonadal hormones, as well as antidiuretic hormone and oxytocin, are all postulated to be regulated, either directly or indirectly, via the hypophyseal gland. Negative feedback signals in the form of circulating levels of these hormones or their precursor trophic hormones are alleged to be monitored by the septal, hypothalamic, or hypophyseal areas. For such a feedback system to function and permit endocrine regulation, the circulating hormones need access to specific sensory cells that are in turn connected to the brain. Many of these molecules, like EP, however, do not easily cross the blood-brain barrier. Furthermore, the necessity of keeping complex molecules out of the brain neuropil seems crucial to maintaining the immunologically privileged status of brain tissue [18]. There have been suggestions in the literature that some of the brain's CVOs are the sites of sensory input for circulating hormones [19,20]. It now seems established that the subfornical organ (SFO) is the site at which angiotensin II acts to induce drinking in the rat [21], and that angiotensin II, like EP, does not penetrate the blood-brain barrier into the brain parenchyma [22]. In a somewhat similar analogy, the brain's emetic center has been located within the dorsal medullary region, adjacent to another CVO, the area postrema (AP). It is postulated that circulating toxins that produce vomiting do so by activating specific chemo-trigger sensory cells within the emetic center and that their site of action is the AP [23].

#### PROSTAGLANDIN E AND THE IMMUNOPRIVILEGED STATUS OF THE BRAIN

If these CVOs do in fact act as the brain's "sensory windows" on the circulation, then they will of necessity come in contact with foreign antigens and other complex molecules that may from time to time enter the circulation during infection and which in the normal course of events produce both immune and inflammatory responses in systemic and peripheral tissues. To prevent this condition, and thereby maintain the brain's immunoprivileged status, it is suggested that the PGE, released within the CVO in response to circulating EP, causes an immunosuppression both within the CVO and perhaps in the brain neuropil immediately surrounding it, which might be exposed to any of the antigenic material that enters the CVO from the circulation. This effect in turn would prevent or reduce potentially damaging immune and inflammatory responses from occurring within the brain tissue itself. In this regard it seems significant that immunoadjuvants, which enhance the body's immune responses to antigens and produce heightened titers of antibody to antigen, also appear to augment the release of PGE in response to the presence of EP at the OVLT and thereby enhance fevers in rats.

There seems ample evidence in the literature to support the idea that PGE has both a



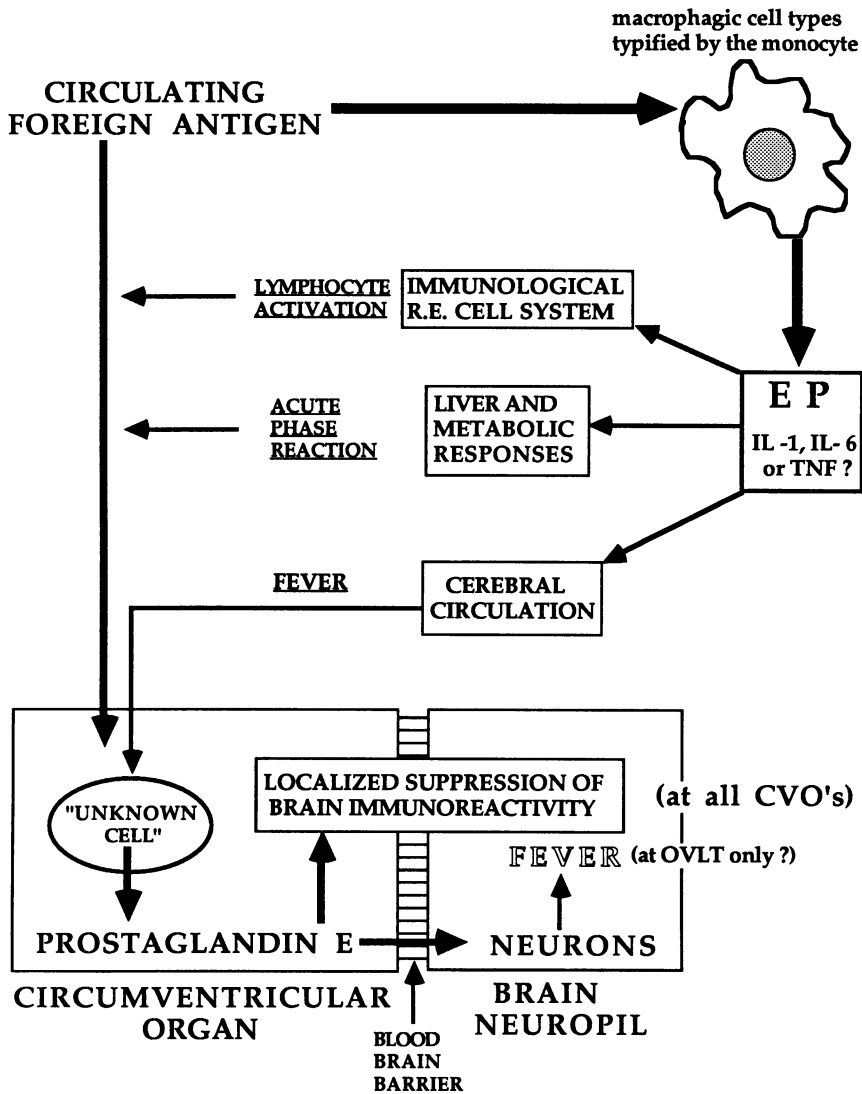


FIG. 7. A hypothesis of the host defense response which incorporates a localized immunosuppressive role for the PGE that is released within CVOs such as the OVLT. This role is in addition to PGE mediation of fever, which may be peculiar to the OVLT, because of its proximity to the PO/AH region.

widespread and profound inhibitory action on most immune system cells [24]. For example, PGE inhibits the mitogenesis of T lymphocytes [25] and inhibits the activity of cytotoxic T cells [26]. It also inhibits the release of lymphokines in response to antigenic challenge [27] and reduces the production of antibodies from B lymphocytes [28]. Furthermore, lysosomal release from polymorphonucleocytes is inhibited by PGE [29], as is phagocytic enzyme release by macrophages [30]. Finally, it has been shown that the release of histamine from mast cells is also suppressed by PGE [31]. Taken in total, all evidence appears to show that PGE, at least *in vitro*, has an immunosuppressive action; however, there is little information about such an action *in vivo*. This

situation exists because the action of PGE is very localized, due to its extremely short half-life *in vivo* and the ubiquity of its catabolic enzymes in all body fluids and tissues. There may appear to be a paradoxical aspect to the idea of PGE acting as a localized immunosuppressive agent, since most aspirin-like drugs that inhibit the formation of prostaglandins also inhibit inflammatory responses when they are administered systemically. Furthermore, non-steroidal anti-inflammatory drugs, when administered systemically, do not appear to have any major effect on whole-body immune responses, but what little *in vivo* evidence there is tends to support the notion that PGE suppresses localized immune responses [32]. This paradox has long been recognized [24], however, and it does not necessarily conflict with the idea of postulating a localized immunosuppressive action by PGE within the CVOs of the cerebral circulation. Indeed, one could speculate that the incidence of Reye's syndrome, which is associated with the administration of aspirin to children suffering otherwise mild infectious or febrile diseases, might be related to such a phenomenon.

Figure 7 summarizes this postulated role for PGE release within the CVO, incorporating it into the larger picture of the host defense responses, including the systemic immune responses, the acute-phase response, and the fever that are produced by cytokines released from a variety of cell types in response to injury or infection.

#### REFERENCES

1. Dinarello CA, Weiner P, Wolff SM: Radiolabelling and disposition in rabbits of purified human leukocytic pyrogen. *Clin Res* 26:522A, 1978
2. Blatteis CM: Neuromodulative actions of cytokines. *Yale J Biol Med* 63:133-146, 1990
3. Stitt JT, Bernheim HA: Differences in endogenous pyrogen fevers induced by IV and ICV routes in rabbits. *J Appl Physiol* 59:342-347, 1985
4. Weindl A: Electron microscopic observations on the organum vasculosum of the lamina terminalis after intravenous injection of horseradish peroxidase. *Neurology* 19:295, 1969
5. Weindl A, Joynt RJ: Electron microscopic observations of the organum vasculosum of the lamina terminalis after intraventricular injection of horseradish peroxidase. *The Anatomical Record* 163:282, 1969
6. Weindl A, Joynt RJ: Ultrastructure of the ventricular walls. *Arch Neurol* 26:420-427, 1972
7. Weindl A, Schwink A, Wetzstein R: Der Feinbau des Gefässorgans der Lamina terminalis beim Kaninchen: I Die Gefässe. *Zeitschrift für Zellforschung* 79:1-48, 1967
8. Weindl A, Schwink A, Wetzstein R: Der Feinbau des Gefässorgans der Lamina terminalis beim Kaninchen: II. Das neuronale und gliale Gewebe. *Zeitschrift für Zellforschung* 85:552-600, 1968
9. Stitt JT: Evidence for the involvement of the organum vasculosum laminae terminalis in the febrile responses of rabbits and rats. *J Physiol* 366:501-511, 1985
10. Blatteis CM, Bealer LS, Hunter WS, Llanos-Q J, Ahokas RA, Mashburn TA: Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. *Brain Res Bull* 11:519-526, 1983
11. Sano Y, Murabe Y: Morphological and functional peculiarities of mesenchymal cells in the pars tuberalis of the pituitary gland. *Cell Tissue Res* 206:171-180, 1980
12. Murabe Y, Nishia K, Sano Y: Cells capable of uptake of horseradish peroxidase in some circumventricular organs of the cat and the rat. *Cell Tissue Res* 219:85-92, 1981
13. Stitt JT, Shimada SG: Immunoadjuvants enhance the febrile responses of rats to endogenous pyrogen. *J Appl Physiol* 67:1734-1739, 1989
14. Stitt JT, Shimada SG: Enhancement of the febrile responses of rats to endogenous pyrogen occurs within the OVLT region. *J Appl Physiol* 67:1740-1746, 1989
15. Hellon RF, Townsend Y: Mechanisms of fever. *Pharmacology and Therapeutics* 19:211-244, 1983
16. Stitt JT: Prostaglandin E as the neural mediator of fever. *Yale J Biol Med* 59:137-149, 1986
17. Tepperman J: *Metabolic and Endocrine Physiology*. Chicago, Medical Year Book, 1962
18. Hart DNJ, Farbre JW: Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissue of rat heart and other tissues, but not brain. *J Exp Med* 153:347-361, 1981
19. Knigge KM, Scott DE, Kobayashi H, Ishii I (ed): *Brain-Endocrine Interaction II: The Ventricular System in Neuroendocrine Mechanisms*. Basel, Karger, 1975

20. Weindl A, Sofroniew MV: Relation of neuropeptides to mammalian circumventricular organs. In Neurosecretion and Brain Peptides. Edited by JB Martin, S Reichlin, KL Bick. New York, Raven Press, 1981, pp 303–320
21. Simpson JB, Routtenberg A: Subfornical organ: Site of drinking elicitation by angiotensin II. *Science* 181:1172–1175, 1973
22. Ramsay DJ, Reid IA: Some central mechanisms of thirst in the dog. *J Physiol* 253:517–525, 1975
23. Borison HL, Brizzee KR: The chemo-trigger zone for emesis. *Proc Soc Exp Biol Med* 77:38–42, 1951
24. Goodwin JS (ed): Prostaglandins and Immunity. Boston, Martinus Nijhoff Publishing, 1985
25. Goodwin JS, Bankhurst AD, Messner RP: Suppression of human T-cell mitogenesis by prostaglandin. *J Exp Med* 146:1719–1734, 1977
26. Droller MJ: Prostaglandins and expression of lymphocyte cytotoxicity. In Prostaglandins and Immunity. Edited by JS Goodwin. Boston, Martinus Nijhoff Publishing, 1985, pp 35–36
27. Wisler RL, Newhouse YG: Inhibition of human B lymphocyte colony responses by endogenous synthesized hydrogen peroxide and prostaglandins. *Cell Immunol* 69:34–45, 1982
28. Gordon D, Bray MA, Morley J: Control of lymphokine secretion by prostaglandins. *Nature* 262:401–402, 1976
29. Marone G, Thomas LL, Lichtenstein LM: The role of agonists that activate adenylate cyclase in the control of cAMP metabolism and enzyme release in human polymorphonuclear leucocytes. *J Immunol* 125:2277–2283, 1980
30. Razin E, Globerson A: The effect of various prostaglandins on plasma membrane receptors and function of mouse macrophages. *Adv Exp Med Biol* 114:415–419, 1979
31. Lichtenstein LM, DiBarnardo R: The immediate allergic response: *in vitro* action of cyclic AMP and other drugs on the two stages of histamine release. *J Immunol* 107:1131–1137, 1971
32. Fantone JC, Kunkel SL, Zurrier RB: Effects of prostaglandins on *in vivo* immune and inflammatory reactions. In Prostaglandins and Immunity. Edited by JS Goodwin. Boston, Martinus Nijhoff Publishing, 1985, pp 123–146