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Target validation and drug discovery for inflammatory diseases

Hideo Kohka Takahashi

Department of Pharmacology, Kinki University, Faculty of Medicine, Osakasayama, Osaka, 589-8511, Japan

Abstract

We have examined to find new factors, which are involved in inflammatory diseases including ischemic diseases. To investigate the mechanism of inflammation, we have proved that monocyte/macropharge plays roles in the up-stream of immune responses. Binding adhesion molecules on monocyte/macropharge and T-cell induces the activation of these cells. It is reported that monocyte/macropharge releases histamine, prostaglandin (PG)E2, interleukin (IL)-18 and high mobility group box-1 (HMGB1). We have found that these mediators regulate immune responses. Therefore, we suggested that histamine, IL-18 and HMGB1 are target for new treatment of inflammatory diseases. In the present review, I explain my findings as the followings.

Key words: adhesion molecule, atherosclerosis, blood-brain barrier, high mobility group box-1, inflammatory diseases, interleukin-18, monocytes, neuron

1. Costimulatory molecules expressed on monocytes and T-cells play important roles in immune responses.

About 20 years ago, interleukin (IL)-18 was originally characterized as an interferon (IFN)-γ-inducing factor in the blood of mice primed with *Propionibacterium acnes* (*P. acnes*) and stimulated with lipopolysaccharide (LPS).[1] IL-18 is reported to be secreted from LPS-activated monocytes/macrophages but also from a wide variety of cells.[2–5] IL-18 is synthesized as a precursor protein that requires cleavage with the IL-1β-converting enzyme/ caspase-1 for activity as in the case of IL-1β.[6,7] Moreover, IL-18 is functionally similar to IL-12 in mediating Th1 response and NK cell activity. IL-18 with IL-12 synergistically produced IFN-γ in T-cells and monocytic cells[8–12] in which IL-12 has been shown to up-regulate IL-18 receptor.[10]

It is well known that the engagement of adhesion molecules expressed on monocytes and T-cells are involved in the activation of T-cells. We found that IL-18 up-regulated intercellular adhesion molecule-1 (ICAM-1) expression on monocytes in human peripheral blood mononuclear cells (PBMCs) and that heterotypic interaction between monocytes and T-cells through ICAM-1/LFA-1 intensified the production of IL-12, IFN-γ, and tumor necrosis factor-α (TNF-α) in PBMCs.[13] The interaction of ICAM-1 with LFA-1 on T-cells generated the costimulatory signal, leading to the enhanced production of IL-12, IFN-γ, and TNF-α. In addition, we found that the interaction between monocytes and T-cells through binding adhesion

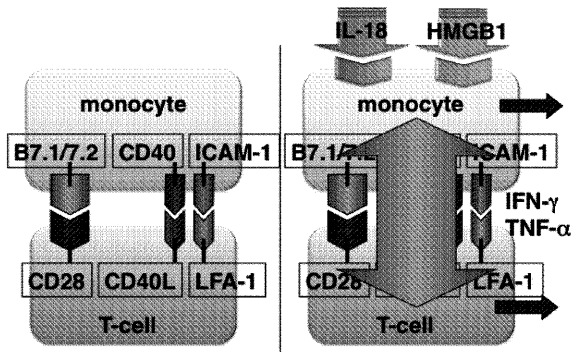

Fig. 1 Adhesion molecules play roles in monocyte-induced activation of T cells.

molecules including B7/CD28 and CD40/CD40L as well as ICAM-1/LFA-1 play roles in the activation of T-cells[14,15] (Figure 1). This means that cytokine cascade initiated by IL-18 has a close relationship to the functional up-regulation of adhesion molecule on monocytes (Figure 1).

2. Histamine induces the production of IL-18 in monocytes.

Histamine is known as a mediator of inflammation and allergic response. In addition to the stable pool of histamine in mast cells and basophilic leukocytes, it is suggested that the presence of histamine with a different dynamic property, called inducible or nascent histamine.[16,17] Many groups reported the induction of histidine decarboxylase, a histamine-synthesizing enzyme, by LPS, cytokines and lymphocyte mitogen in macrophages[18] and T-cells,[19] and in many peripheral tissues.[20,21] Although histamine may be one of the regulators of immune response,[22–25] the estimated functional roles of histamine as immunomodulator are often controversial probably due to the differences in the cell preparations used and the complexity of the involvement of histamine in immunomodulation.[26]

We reported that histamine stimulated the production of IL-18 and IFN-γ and inhibited the production of IL-2 and IL-10 in human PBMCs, whereas histamine did not induce the production of IL-12 at all.[27] The stimulatory or inhibitory effects of histamine on cytokine production were all antagonized by H2-receptor antagonists, ranitidine and famotidine, but not by H1- and H3-receptor antagonists. Selective H2-receptor agonists, 4-methylhistamine and dimaprit, mimicked the effects of histamine on cytokine production, indicating that the action of histamine depends on the stimulation of H2-receptors. All effects of histamine on cytokine responses were also inhibited by the presence of either anti-IL-18 Ab or IL-1-converting enzyme/caspase-1 inhibitor, indicating that the histamine action is dependent on mature IL-18 secretion and that IL-18 production is located up-stream of the cytokine cascade activated by histamine. Recombinant human IL-18 induced the production of IL-12 and IFN-γ and inhibited the production of IL-2 and IL-10. IL-18-induced IFN-γ production was inhibited by anti-IL-12 Ab, indicating the marked contrast of the effect of histamine. Thus histamine plays important roles in inducing Th1 cytokine production in PBMCs and is quite unique in triggering IL-18-initiating cytokine cascade without inducing IL-12 production.

The proliferation of the precursor of both cells and the production of IgE Abs are stimulated by Th2 cytokines such as IL-4, which are secreted by stimulated mast cells and basophils.[28,29] Histamine is a storage amine of mast cells and basophils, indicating that the effects of histamine on Th1 cytokine production function as a negative feedback on excessive Th2 response. Ii is also reported that IL-18 with IL-3 stimulates histamine release from cultured basophils,[30] suggesting the presence of a positive feedback system between histamine release and IL-18 secretion under certain conditions. Histamine plays much more diverse effects on immune cells than expected by modulating the cytokine production and to be a factor enabling the cross-talk between Th1 and Th2 cells.

3. Histamine inhibits IL-18-induced monocyte activation.

There is controversy on the effects of histamine on IFN-γ production in human PBMCs among earlier works.[31] It is reported that histamine increases IL-10 production in human whole blood culture stimulated by LPS,[32] suggesting the differential effects of histamine under the conditions with varied monocyte stimulation.

We demonstrated that histamine inhibited the IL-18-induced expression of ICAM-1 on monocytes and production of IL-12, IFN-γ, and TNF-α in PBMCs, whereas IL-18-inhibited IL-10 production was reversed by histamine.[14] The modulatory effects of histamine on ICAM-1 expression and cytokine production were antagonized by famotidine but not by *d*-chlorpheniramine and thioperamide, and were mimicked by selective H2-receptor agonists but not by H1- and H3-receptor agonists, indicating the involvement of H2-receptors in histamine action. The inhibition of IL-18-induced IFN-γ by histamine was ascribed to the strong inhibition of IL-12 production by histamine. Thus, histamine operates the negative feedback mechanism against IL-18-activated cytokine cascade through the strong inhibitory effect on ICAM-1 expression and IL-12 production in monocytes, contributing to the formation of diverse pattern

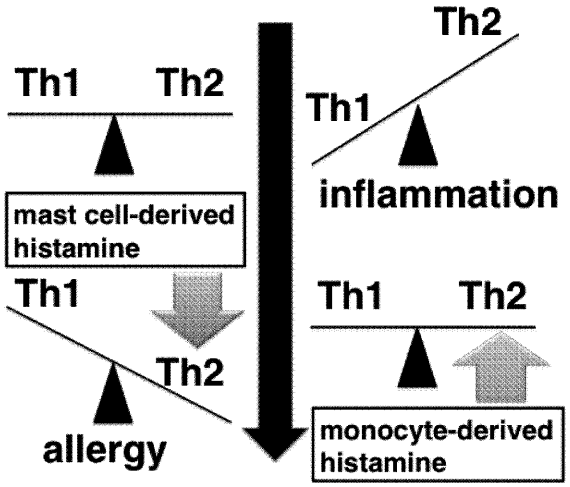

Fig. 2 Histamine regulates Th1/Th2 balance.

of cytokine activation from Th1 to Th2, depending on the monocyte/macrophage activation and cytokine environment (Figure 2).

4. The other autacoid, PGE2 inhibits the activation of monocytes via prostanoid EP2-and EP4-receptors.

It is known that the stimulation of prostaglandin (PG)E2-receptors, EP2 and EP4, as well as that of histamine H2-receptors activates the cAMP-dependent signaling pathways. We investigated the effects of PGE2 on the expression of ICAM-1, B7.1, and B7.2 on monocytes in IL-18-stimulated PBMCs.[15] PGE2 inhibited ICAM-1 and B7.2 expression elicited by IL-18 in PBMCs. We examined the involvement of four subtypes of PGE2 receptors, EP1, EP2, EP3, and EP4, in the modulatory effect of PGE2 on ICAM-1 and B7.2 expression elicited by IL-18, using subtype-specific agonists. EP2-receptor agonist inhibited IL-18-elicited ICAM-1 and B7.2 expression with a potency slightly less than that of PGE2, while EP4-receptor agonist was much less potent than PGE2. However, EP1-receptor agonist and EP3-receptor agonist showed no effect on IL-18-elicited ICAM-1 or B7.2 expression. These results indicated that EP2- and EP4-receptors were involved in the action of PGE2. Dibutyryl cAMP and forskolin down-regulated ICAM-1 and B7.2 expression in IL-18-stimulated monocytes. Because EP2- and EP4-receptors are coupled to adenylate cyclase, we suggest that PGE2 down-regulates IL-18-induced ICAM-1 and B7.2 expression in monocytes via EP2- and EP4-receptors by cAMP-dependent signaling

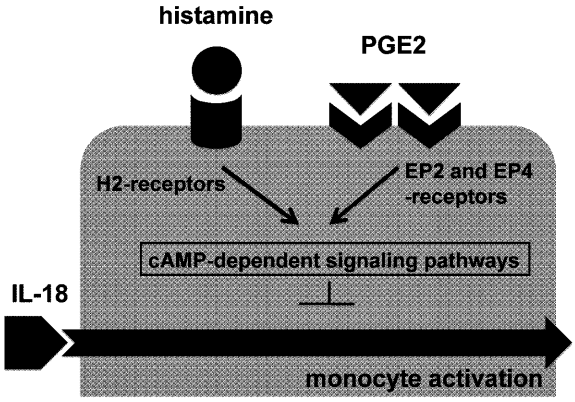

Fig. 3 cAMP-dependent signaling pathways.

pathways. Together with these results, PGE2 may modulate the immune response through regulation of the expression of particular adhesion molecules on monocytes through the stimulation of EP2- and EP4-receptors and the cAMP-dependent signaling pathways (Figure 3).

5. Histamine inhibits hepatitis via histamine H2-receptors.

Fulminant hepatic failure is pathologically characterized to be caused by diffuse intrahepatic infiltration by inflammatory cells with massive multilobular necrosis. Intrahepatic infiltrates are composed predominantly of T lymphocytes, and Fas ligand expression is found in the areas with lymphocyte infiltration.[33] It is reported that the IL-18-inducing stimulation, heat-killed *P. acnes* followed by a challenge with a low dose of LPS,[1] induces acute and massive liver injury, mimicking fulminant hepatic failure.[34] In IL-18 knockout mice, the development of hepatitis is markedly reduced, accompanying a decrease in the production of TNF-α and the expression of Fas in the liver.[35]

To prove the inhibitory effect of H2-receptors stimulation on inflammatory response in *in vivo* model, we examined a functional role of inducible histamine in the protection against hepatic injury and lethality in *P. acnes*-primed and LPS-induced hepatitis, using histidine decarboxylase knockout and H2-receptor knockout mice.[36] LPS challenge after *P. acnes* priming increased histidine decarboxylase activity in the liver of wild-type mice, associated with a marked elevation of histamine turnover. Histidine decarboxylase-like immunoreactivity was observed in CD68-positive Kupffer cells/macrophages. Treatment of wild-type mice with H2-receptor

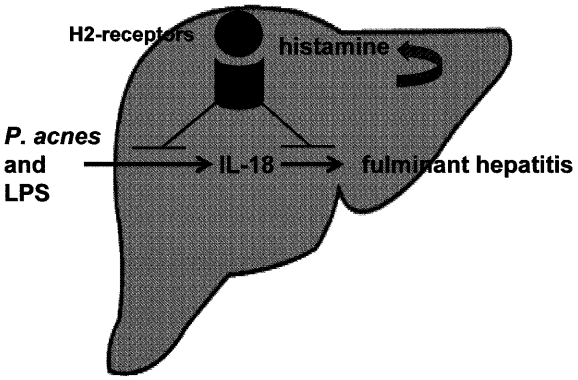

Fig. 4 Histamine inhibits hepatitis.

antagonists, famotidine or ranitidine, augmented hepatic injury and inhibited the survival rate significantly. Although the same dose of *P. acnes* and LPS induced severe hepatitis and high lethality in histidine decarboxylase knockout and H2-receptor knockout mice, the former were rescued by the subcutaneous injection of histamine.

Immunohistochemical study supported the protective role of histamine against the apoptosis of hepatocytes. Histamine suppressed the expression of IL-18 and TNF-α in the liver, reducing plasma levels of cytokines including IL-18, TNF-α, IL-12, IFN-γ, and IL-6. Together with these findings, endogenously produced histamine in Kupffer cells/macrophages may play a very important role in preventing excessive innate immune response in endotoxin-induced fulminant hepatitis through the stimulation of H2-receptors. Therefore, it is suggested that histamine has therapeutic potential for the treatment of endotoxin-induced hepatitis through the stimulation of H2-receptors (Figure 4). However, the stimulation of H2-receptors may produce harmful side effects.

6. The action of other monocyte-initiated mediator, HMGB1 inhibits cerebral infarction.

Recently, it is proposed that inflammatory response plays roles in ischemic disease. In ischemic stroke, interruption of blood flow induces neuronal death in the ischemic core as a result of the inability to maintain membrane ion gradients in neurons, excitotoxicity due to elevated glutamate levels and disruption of the blood-brain barrier (BBB). The penumbra, surrounding the core, receives a relatively low blood supply and develops time-dependent inflammatory responses that can be deleterious to the surviving neurons, indicating that this region can be reversibly rescued and thus could be a target for drug treatment. A diversity of neuroprotective candidate drugs targeting varieties of factors in patients with brain ischemia is subjected to preclinical and clinical studies.[37,38] Despite these extensive efforts, however, an effective therapy has not yet been successfully established.

High mobility group box-1 (HMGB1), an architectural nuclear protein, exhibits an inflammatory cytokine-like activity in the extracellular space. The cytokine-like activity has shed new light on the role of nuclear proteins and promoted the studies on roles of this unique factor in different diseases that are accompanied by inflammatory responses.[39–43] Extracellular HMGB1 is secreted from activated monocytes/macrophages and released from the nuclei of necrotic cells[44,45] (Figure 5). In monocytes/macrophages, HMGB1 is reported to stimulate the production of IL-1, TNF-α, IL-6, and IL-8,[46] induce iNOS,[47] and stimulate chemotaxis.[48] We found that HMGB1 induced the expression of ICAM-1, B7 and CD40, and the production of TNF-α and IFN-γ in human PBMCs (submitting data) (Figure 1). HMGB1 did not induce IL-18 and IL-12 production, whereas anti-IL-18 and anti-IL-12 Ab had no effect on the actions of HMGB1, suggesting that the effects of HMGB1 may be independent from IL-18 and IL-12 actions. Based on these data, we demonstrated the involvement of HMGB1 in inflammatory disease.

In the central nervous system, HMGB1 is

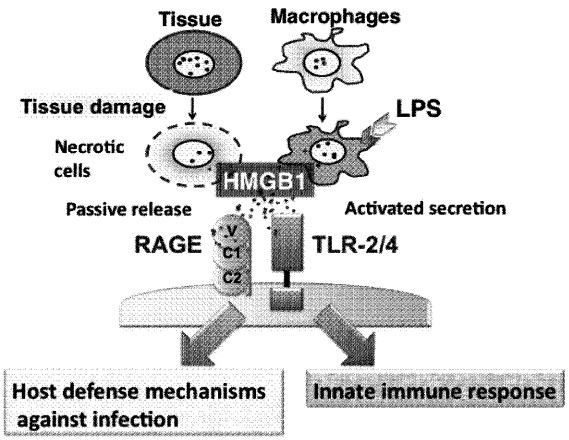

Fig. 5 HMGB1 and receptors for HMGB1.

shown to inhibit glutamate transport by glial glutamate-aspartate transporter 1 using the mouse glial membrane preparation,[49] suggesting a contribution of HMGB1 to the elevation of excitotoxic glutamate in ischemic brain. Because therapeutically injected[50–52] and endogenous t-PA[53] have been implicated in the activation of matrix metalloproteinase-9 (MMP-9) during brain ischemia, HMGB1 may enhance the disruption of BBB structure through activation of MMP via t-PA.

We reported that treatment with neutralizing anti-HMGB1 monoclonal antibody (mAb) remarkably ameliorated brain infarction induced by 2-h occlusion of the middle cerebral artery in rats, when the mAb was administered after the start of reperfusion.[54] Consistent with the 90% reduction in infarct size, the accompanying neurological deficits in locomotor function were significantly improved. Anti-HMGB1 mAb inhibited the increased permeability of the BBB, the activation of microglia, the expression of TNF-α and iNOS, whereas the mAb suppressed the activity of MMP-9. Immunohistochemical point of view, HMGB1 in the cell nuclei was decreased in the affected areas, suggesting the release of HMGB1 into the extracellular space. Together with these results, HMGB1 may play roles in the development of brain infarction through the amplification of plural inflammatory responses in the ischemic region (Figure 6). Moreover, HMGB1 can be an outstandingly suitable target for the treatment.

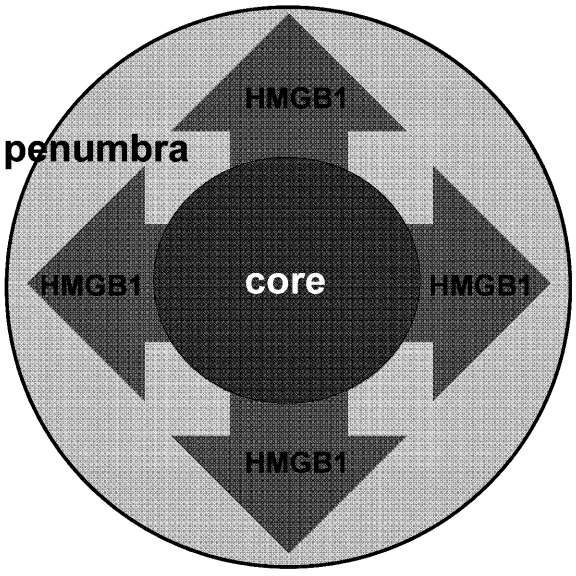

Fig. 6 HMGB1 induces progression of penumbra area.

7. Anti-HMGB1 mAb inhibits the disruption of the blood-brain barrier (BBB) in cerebral infarction.

In the previous study, we found that intravenous injection of neutralizing anti-HMGB1 mAb provides a novel therapeutic strategy for ischemic stroke, however little is still known the mechanism of the mAb action. Then, we focused on the protective effects of the mAb on the marked translocation of HMGB1 in the brain, the disruption of the BBB, and the resultant brain edema[55] (Figure 7). HMGB1 was time-dependently translocated and released from neurons in the ischemic rat brain. When BBB permeability was measured by T2-weighted MRI, the intravenous injected mAb reduced the edematous area. Transmission electron microscope observation, which was to investigate ultrastructure of the BBB unit, revealed that the mAb strongly inhibited astrocyte end feet swelling, the end feet detachment from the basement membrane, and the opening of the tight junction between endothelial cells.

The *in vitro* BBB system was used to study the direct effects of HMGB1 in BBB components. Recombinant HMGB1 increased the permeability of the BBB with morphological changes in endothelial cells and pericytes, which were inhibited by the mAb. Moreover, the anti-HMGB1 mAb facilitated the clearance of serum HMGB1. These results indicated that the anti-HMGB1 mAb could be an effective therapy for brain ischemia by inhibiting the development of brain edema through the protection of the BBB and the efficient clearance of circulating HMGB1.

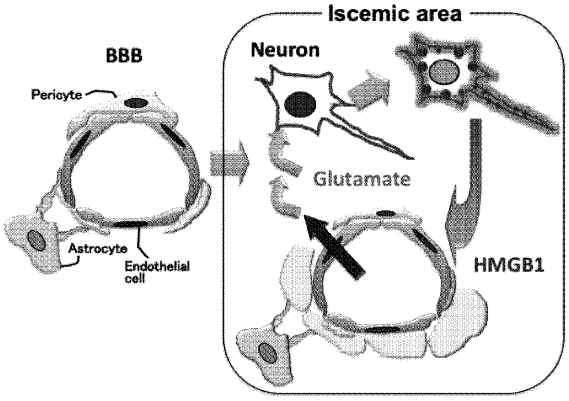

Fig. 7 Involvement of HMGB1 in disruption of the BBB.

8. HMGB1 neutralization reduces development of diet-induced atherosclerosis.

It is known that patients with atherosclerosis show the high incidence of brain ischemia. HMGB1 is reported to express in atherosclerotic lesions, however its pathophysiological role in atherosclerosis is still unknown. We investigated the involvement of HMGB1 in the development of atherosclerosis in ApoE knock out (ApoE-/-) mice.[56] Using ApoE-/- mice fed a high fat diet, the effects of anti-HMGB1 mAb on lesion size and immune cell accumulation were determined. HMGB1 expressed in atherosclerotic lesions of ApoE-/- mice, and the mAb attenuated atherosclerosis and macrophage accumulation. Moreover, the mAb also reduced CD4+ T-cells. It is suggested that HMGB1 shows proatherogenic effects augmenting lesion development by stimulating macrophage migration as well as the accumulation of immune and smooth muscle cells.

9. Conclusion

From pharmacological viewpoint, we should find mediators, which play roles in inflammatory diseases. Monocyte/macropharge is regard as an important factor in immune responses. We have found that the investigation about monocyte/macropharge-initiated mediators, IL-18 and histamine, was a novel way of development of treatment for inflammatory diseases. On the other hand, the investigation indicated that the unknown mediator was located up-stream of the IL-18- and histamine-induced immune responses.

Recently, HMGB1 is recognized as a representative of damage-associated molecular patterns (DAMPs).[57,58] It is reported that HMGB1 contributes to pathophysiology of sepsis,[59] ARDS,[60] arthritis[61] and acute rejection.[62] Ligands for receptor for advanced glycation end products (RAGE), S100 protein and advanced glycation end products (AGEs) as well as HMGB1, are suggested to play roles in DAMPs. Therefore, the ligands become the center of considerable attention. Experiments using the mAb against ligands for RAGE will provide a novel therapy for inflammatory diseases. Target validation and drug discovery should be continued.

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