Review

Suppression of Response to Foreign Substances by Intestinal Macrophages

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Abstract. *Macrophages play an important role in the maintenance of homeostasis by changing their function according to the tissue and environment everywhere in the body. We have proposed that intestinal macrophages, which exist in the front line receiving environmental information, have an important function in forming a macrophage network for biophylaxis. In this review, we introduce intestinal macrophages as an example of the highly plastic and flexible cells adaptable to environmental information. Intestinal macrophages are hyporesponsiveness to foreign substances, especially lipopolysaccharide (LPS), and less expression of CD14 and TLR4/MD-2, receptors for LPS. However, those proteins expression was observed in the cytoplasm of intestinal macrophage. We also found that intestinal macrophages treated with IgA could restore in response to LPS. In conclusion, intestinal macrophages possess the plasticity to respond sensitively to change in their environment and are considered to be involved inflammatory bowel disease development.*

Hyporesponsiveness to LPS and CD14 Expression in Intestinal Macrophages

Macrophages are cells which infiltrate into tissue as monocytes and change in character according to the tissue environment. As macrophages are present in every tissue, they are considered to form a network, a macrophage network, in which environmental information is transferred among neighboring macrophages thus relaying information to the whole body. This network is considered to play an important role in the maintenance of homeostasis (1). In particular, as macrophages residing in the intestine which have the most extensive contact with the exterior environment, and are exposed to large quantity of foreign substances, are considered to be very important in macrophage network formation. However, intestinal macrophages are not activated by foreign substances and the information transfer to other cells are not known.

Hyporesponsiveness of intestinal macrophages to foreign substances, especially LPS, is thought to be ascribed to non-expression of CD14, a receptor for LPS. In contrast, in an inflamed intestine such as in Crohn's disease or ulcerative colitis, CD14 is expressed and macrophage responsive to LPS is present. Such macrophages are reported to be derived from migrated monocytes (2, 3), and also from resident macrophages (4). Some factors present in the intestine [such as interleukine-10 (IL-10) and transforming growth factor-β (TGF-β)] have been studied in relation to the regulation of expression of intestinal macrophages (5), but details of the intracellular regulatory mechanism of macrophage expression are not clear.

Analysis of CD14 mRNA Expression in Intestinal Macrophages

Many papers have reported that CD14 protein expression was not observed on the cellular membrane of intestinal macrophages and this is considered to be characteristic (6-8). It has also been reported that CD14 mRNA is expressed

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at an extremely low level in intestinal macrophages derived from the small intestine compared with peripheral monocytes (9) and that it is expressed at the same level as monocytes in intestinal macrophages derived from the large intestine (10). Monocytes are precursors which differentiate into tissue macrophages. We have compared the difference in CD14 expression in intestinal macrophages from the large intestine with that in other tissue macrophages (alveolar macrophages and peritoneal macrophages) (11). As measured by real-time PCR the CD14 mRNA expression in the intestinal macrophages was lower than that in the peritoneal macrophages but higher than that in the alveolar macrophages. As to the expression of intracellular signal transfer molecules (Myeloid differentiation factor 88 (MyD88), Toll/IL-1 receptor (TIR) domain-containing adapter protein (TIRAP), IL-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6), and TIR domain-containing adapter inducing interferon-β (TRIF)), no difference could be confirmed between the three kinds of macrophages. The alveolar macrophages respond to LPS as much as, or more than the peritoneal macrophages. Therefore, the intestinal macrophages could be said to express sufficient CD14 mRNA to respond to LPS. With stimulation by LPS, the expression of CD14 mRNA and that of tumor necrosis factor (TNF) was significantly augmented both in the alveolar macrophages and the peritoneal macrophages, which have responsiveness to LPS, while no change was observed in the mRNA expression of the intracellular signal transfer molecules. The same change, however, was not observed with intestinal macrophages. This result suggested that the hyporesponsiveness to LPS of intestinal macrophages may be ascribable to the state of the CD14 protein, which was expressed but incapable of recognizing LPS.

Analysis of CD14 protein Expression by Intestinal Macrophages

Since analysis of CD14 mRNA expression did not explain its lack of expression as a membrane protein, we next studied the possibility of suppression of CD14 protein expression. In general, expression of protein on the cellular membrane after transcription from mRNA, requires several stages such as translation by the ribosome, post translational modification in the endoplasmic reticulum or Golgi body and transfer to the appropriate place by a transport vesicle. CD14 protein was observed, by immunostaining and flow cytometry analysis to be present in the cytoplasm of intestinal macrophages (11) and also Toll like receptor 4 (TLR4)/MD-2 was expressed in the cells.

Gp96, a chaperone protein, has been reported to suppress the expression of TLR4/MD-2 in the Golgi body (12). Also, intracellular TLR4/MD-2 in intestinal epithelial cells has been reported to be capable of binding with LPS in the cytoplasm. In intestinal macrophages in which the expression of CD14 was restored a response to LPS was confirmed (4).

All these reports suggested the strong possibility that CD14 in intestinal macrophages possesses the functional ability to respond to LPS. Thus the presence of a suppressive mechanism against CD14 transport, similar to that of gp96 is a possibility in intestinal macrophages.

Recovery of Responsiveness to LPS by Treatment with IgA

In inflammatory bowel disease, the dysfunction of intestinal macrophages has been pointed out, but it is not clear whether the responsiveness to LPS remains unsuppressed or whether it is restored and contributes to the onset of the disease. An explanation of the mechanism of onset of inflammatory bowel disease by intestinal macrophages would give an important basis for establishing a new therapy or preventive method. We found that intestinal macrophages treated with IgA could recover TNF producing activity in response to LPS (13). IgA is thought to have a role similar to the immunocomplex with on antigen. Although detail of the mechanism is unknown, there is a possibility that expression of CD14, as a receptor to LPS was restored since responsiveness to LPS was recovered by the stimulation with the IgA. In dendritic cells, it has been reported that MHC-class II molecules synthesized in the cytoplasm move to the lysosome or the endosome and stay there when there is no stimulation, but that expression on the cellular membrane is induced by a particular signal for maturation (14). Thus not all the membrane proteins produced were expressed on the cellular membrane, but a control mechanism exists whereby a certain signal induces such expression. We consider that CD14 and other LPS receptors accumulate within intestinal macrophages and their expression on the cellular membrane is induced by a certain signal from the IgA receptor and thus responsiveness to LPS is restored.

The Biological Significance of the Recovery of Responsiveness to LPS by Intestinal Macrophages with Stimulation by IgA

IgA is an immunoglobulin, present mostly in mucosal tissue which exists as a form of monomer, mIgA1 and mIgA2, a dimmer, dIgA or secretory IgA, in which a secretory component (SC) is bound to the IgA (15). IgA in serum is mainly mIgA1, and is present as polymeric IgA (pIgA) of mIgA2 in mucus. Also secretory IgA in the intestine is secreted from two sources in mice (16, 17), 75% is produced in a T cell dependent manner by B2 cells in mucosal lymphoid tissue such as Peyer's patches and the remaining

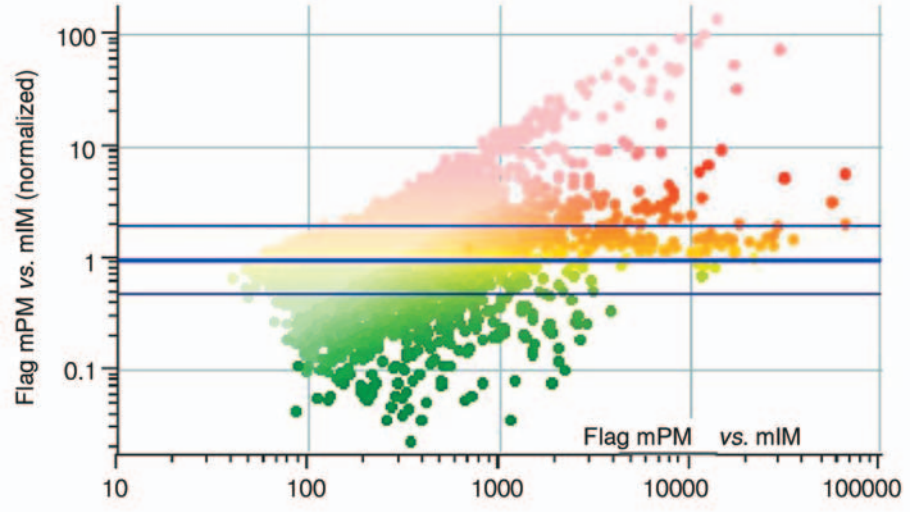

Figure 1. *Difference in gene expression by intestinal macrophages and peritoneal macrophages as observed in DNA array. RNA was extracted from intestinal macrophages and peritoneal macrophages of normal mice, and difference in mRNA expression was compared. Gene Chip® Mouse Genome 430 2.0 Array (Affimetrix, Inc. Japan) was used to analyze DNA array. X axis: quantity of expression of mRNA, Y axis: proportion in quantity of mRNA expressed by intestinal macrophages (mIM) to that by peritoneal macrophages (mPM).*

25% is produced by B cells present in the lamina propria of the intestine. IgA on the mucosal surface works as a mucosal barrier to defend against bacterial or viral infection. It also neutralizes local inflammation caused by pathogenic components like LPS (18). As a receptor recognizing IgA, Fc receptor for IgA (FcαRI, CD89) is well-known in macrophages (19). After binding with CD89, IgA bound with antigen induces antibody dependent cell-mediated cytotoxicity (ADCC), phagocytosis or antigen presentation (20). Surprisingly, CD89 is not expressed in intestinal macrophages (5, 9, 21). Furthermore, CD89 dose not exist in the genome in mice (18, 22). From these results, it is appropriate to think that an IgA receptor other than CD89 suppresses expression in intestinal macrophages.

Preliminary Study of the Expression of IgA Receptors by DNA Array

Receptors recognizing IgA other than CD89, such as Fc receptor for IgA and IgM (Fcα/μR), polymeric Ig receptor (pIgR), CD71, asialoglycoprotein receptor (ASGPR), and so on are known (15, 18). Fcα/μR is a receptor binding with IgA and IgM, and expressed on B cells and or macrophages (23, 24). pIgR is mainly expressed on epithelial cells and works to transport pIgA to the mucosal surface (25, 26). CD71 is mainly expressed in the monocytes of the fetus and is not expressed in the monocytes or polykaryocytes of an adult (18). ASGPR is expressed in hepatocytes and participates in the metabolism of serum IgA (27-29). Fcα/μR and pIgR are known to be expressed in the intestine but there has been no report yet of expression in intestinal macrophages. A comprehensive method for the analysis of the involvement of these related molecules, would be useful. We have made a comparative analysis by DNA array of intestinal macrophages using peritoneal macrophages as control. Out of about 20,000 genes, about 740 were found to be expressed at a higher level in intestinal macrophages than in peritoneal macrophages and about 800 were found to be expressed at a lower level in intestinal macrophages than in peritoneal macrophages (Figure 1). Among the IgA receptors, the expression of pIgR was observed to be remarkably higher than the other receptors in intestinal macrophages (Table I). pIgR is known to be expressed in epithelial cells of the intestine and functions to transport locally formed pIgA from the lamina propria into the mucosal secretions or as an antibacterial peptide to eliminate pathogens present in the intestine (25). The binding of pIgR with an IgA dimmer leads to tyrosine phosphorylation of

Table I. *IgA receptor mRNA expression by mouse intestinal macrophages in proportion to mouse peritoneal macrophages.*

	Gene	Normalized
IgA receptors	polymeric immunoglobulin receptor	12.80
	asialoglycoprotein receptor 1	1.14
	asialoglycoprotein receptor 2	1.07
	transferrin receptor	1.12
	transferrin receptor 2	0.97
	Fc receptor, IgA, IgM, high affinity	1.02

phosphatidyl inositol-specific phospholipase Cγ1, production of inositol 1,4,5-triphosphate (IP3), activation of protein kinase C (PKC) and augmentation of the intracellular concentration of Ca^{2+} (30, 31). Therefore, for the analysis of the co-relationship between IgA and pIgR in intestinal macrophages, it is necessary to confirm the presence of IgA or pIgR proteins by staining or to examine methods to block the signal pIgR pathway by using the PKC inhibitor (Staurosporine and so on). Like IgA, pIgR is not reported to affect the regulation of cellular function. Therefore, if pIgR is proved to be expressed in intestinal macrophages and has the function of controling expression of CD14 through binding with IgA, a new function of pIgR or IgA may have been discovered.

Environmental Adaptability of Macrophages

Intestinal macrophages are said to have no other response than phagocytosis of foreign bodies, but the present study has confirmed that they possess molecular responsiveness to LPS following specific stimulation, such as by IgA. From this result, intestinal macrophages are considered originally not to lack responsiveness to foreign substances, but that function is thought to be strictly suppressed by various factors present in the intestine. The number of patients with inflammatory bowel disease such as Crohn's disease or ulcerative colitis is increasing. The cause of these diseases is still unclear, but intestinal TNF producing macrophages are considered to be involved, as therapy with anti-TNF antibody is extremely effective against Crohn's disease. Developing this idea, we consider that suppressive factors in the intestine are disrupted by sudden dietary change, leading to the activation of TNF productivity in intestinal macrophages, which possess the plasticity to respond sensitively to changes in their environment and cause inflammatory bowel diseases.

Conclusion

Intestinal macrophages are known to be hyporesponsive to LPS. On the other hand, our studies showed that responsiveness to LPS was recovered by treatment with IgA. As CD14, a LPS receptor, is expressed in intestinal macrophages, the treatment with IgA is thought to induce its transport to the cell membrane and to restore its ability to bind LPS. CD89, a major IgA receptor, is however not expressed in intestinal macrophages. A study of other receptors which receive signals from IgA suggested the involvement of pIgR. There have been no reports that IgA or pIgR possess a control function in other cells. Therefore, if the recovery of responsiveness to LPS brought about by IgA in intestinal macrophages is confirmed to be induced through pIgR, it may reveal a new function not only of intestinal macrophages but also of IgA or pIgR. Also, the elucidation of the mechanism regulating response to foreign substances by intestinal macrophages would be useful for the development of foods with specific functions or the establishment of preventive or therapeutic treatments for inflammatory bowel diseases.

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