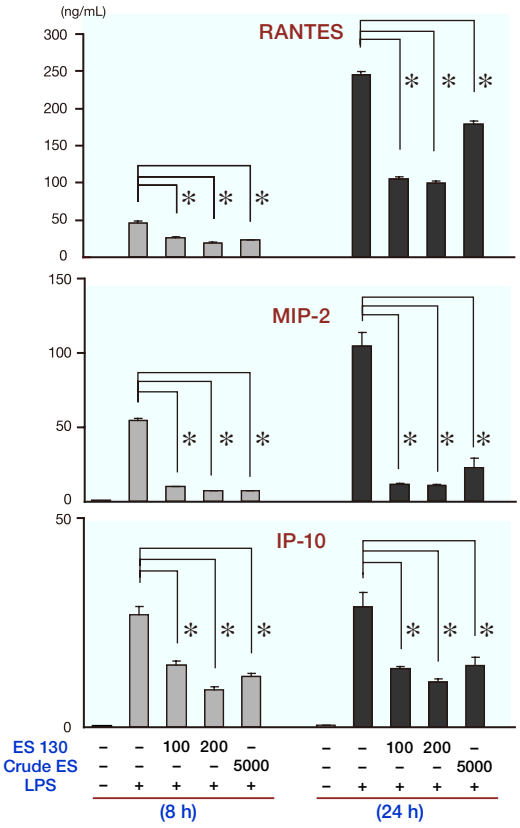

Fig. 5. ES130 suppressed the chemokine production significantly in the incubation medium of LPS-stimulated macrophages. Peritoneal macrophages were left untreated or treated with ES130 (100 or 200 ng/mL) or crude ES (5000 ng/mL) for 24 h, and then stimulated with LPS (100 ng/mL) for 8 h or 24 h. The incubation medium was obtained, and the levels of 3 chemokines, RANTES, MIP-2 and IP-10 were quantified by enzyme-linked immunosorbent assay. Data are expressed as the mean ± SE (n = 3). *$P < 0.005$. ES, excretory/secretory; IP-10, interferon-inducible protein 10 kDa; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T cell expressed and secreted; RT, reverse transcriptase.

RANTES in the medium of macrophages stimulated with LPS for 8 h increased nearly 5 times in the medium after LPS stimulation for 24 h. Although the mRNA expression of RANTES in macrophages stimulated with LPS for 8 h or 24 h was not suppressed significantly by ES130 or crude ES products (Fig.4), the same amount of ES130 and crude ES products suppressed the RANTES chemokine levels in the medium of these macrophages significantly (Fig. 5).

Discussion

Parasite survival may depend on the ability of the parasite to modulate the host immune response by the release of immunomodulatory molecules that protect the organism (Ramaswamy et al., 1995; Goodridge et al., 2001, 2005). We purified an immunosuppressive factor (ES130) from ES products of plerocercoids in the present study and found that ES130 inhibits the gene expression of IL-1β and TNF-α as well as plerocercoid-immunosuppressive factor reported by Kina et al. (2005). Besides, ES130 suppressed the gene expression of 3 chemokines, MIP-2, IP-10 and RANTES, in LPS-stimulated macrophages.

In monocytes or macrophage cell lines, LPS has been reported to activate p42/44 (ERK) mitogen-activated protein kinase (MAPK) and JNK as well as the p38 MAPK cascade (Hambleton et al., 1996; Carter et al., 1999). Transcription of c-Fos is upregulated by MAPKs, and members of the Fos family dimerise with Jun to form the AP-1 transcription factor, which upregulates transcription of a diverse range of genes in macrophages.

MIP-2 plays an important role in the recrutment of neutrophils. The MIP-2 promoter is transcriptionally activated in a macrophage cell line RAW 264.7 by LPS. By deletion analysis of the MIP-2 promoter region, Kim et al. (2003) showed that NF-κB and AP-1 binding sites are essential for LPS-induced MIP-2 gene expression. The Sp-1 binding element is an important determinant of MIP-2 promoter activity, and NF-κB, c-Jun and Sp-1 can functionally cooperate to elicit maximal activation of the promoter (Lee et al., 2005).

The p38 MAPK intracellular signaling pathway plays a central role in regulating a wide range of inflammatory responses in many different cells. In vitro exposure to a novel p38 MAPK inhibitor (M39) blocked MIP-2 release from LPS-stimulated murine and human neutrophils and macrophages, and eliminated migration of murine neutrophils toward the chemokines MIP-2 and KC (Nick et al., 2000). We previously showed that

the ES products from the plerocercoids reduce the phosphorylation of MAPK, particularly extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 MAPK (Dirgahayu et al., 2002, 2004), which may attribute to the suppression of MIP-2 mRNA expression. Besides MAPKs it has been emphasized the importance of NF-κB for the induction of MIP-2 in LPS-activated monocyte-macrophage lineage. However, we found that the ES products do not affect LPS-induced nuclear translocation of NF-κB (Dirgahayu et al., 2004).

Shin et al. (1994) showed the LPS-response elements in RANTES chemokine gene. Promoter regions of RANTES contain an LPS-response element, which is composed of 2 sequences, one corresponding to an AP-1 half site and another resembling a portion of interferon (IFN)-stimulated response element (ISRE). There are a number of proteins capable of binding to AP-1 sites. They belong to the Jun-Fos family, ATF-CREB family, and NF-E2. Monomeres of these proteins have negligible affinity toward the AP-1 site, in contrast to their homo- or heterodimers. The results from electrophoretic mobility shift assay demonstrarated the presence of c-Jun and CREB in the protein-LER complex (Shin et al., 1994). However, the ISRE-like motif in RANTES does not have ISRE activity.

Compared to MIP-2 and IP-10, the mRNA expression of RANTES in macrophages stimulated with LPS for 8 h or 24 h was not inhibited by ES130 or crude ES products, while the same amount of ES130 and crude ES products suppressed the RANTES chemokine levels in the medium of macrophages significantly stimulated with LPS for the same amount of time. The production of RANTES chemokine protein can be suppressed by nociceptin in both primary $CD14^+$ human monocyte and monocyte-like cell lines. However, nociceptin does not appear to regulate the expression of the chemokine at the level of transcription, as RANTES mRNA levels following nociceptin treatment of monocytes were essentially normal (Kaminsky and Rogers, 2008). Although the mechanism of RANTES chemokine regulation by ES130 is as yet unknown, it is supposed that ES130 plays a role in inhibiting the translation, as noiciceptin does.

In LPS-stimulated macrophages, the IP-10 expression is mediated by the intermediate production of IFN-β in MyD88-independent pathway (Kawai et al., 2001; Toshchakov et al., 2002). IFN-β activates IFN stimulated gene factor 3, a trimeric complex composed of signal transducer and activator of transcription (STAT) 1, STAT2 and IFN regulatory factor-9/p48 (Darnell et al., 1994). Consistent with this, LPS-induced expression of IP-10 is severely impaired in macrophages from STAT1-deficient or IFN-$\alpha/\beta R^{-/-}$ mice (Ohmori and Hamilton, 2001). LPS-induced transcriptional activation of the IP-10 gene in a macrophage cell line is mediated by regulatory sequences found in the region between –243 and –105 including an ISRE and 2 κB sites, and the cooperative interaction between ISRE and κB elements is essential for the IP-10 gene expression (Ohmori and Hamilton, 2001). As the IFN-β gene expression in LPS-stimulated macrophages is suppressed by crude ES products (data not shown), and this suppression is supposed to be one of the mechanisms in inhibiting the IP-10 gene expression by ES130.

The present results suggest that ES130 attenuates inflammation around the plerocercoids through suppression of the chemokine gene expressions and chemokine production. Its mechanism needs to be further investigated.

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