(data not shown). Thus, the adhesion to fibrinogen enhanced the proliferation mast cells in the presence of SCF but did not prevent the apoptosis of mast cells in the absence of cytokines.

Endocytosis of fibrinogen into mast cells via integrin $\alpha_{IIb}\beta_3$

We analyzed the interaction of integrin $\alpha_{IIb}\beta_3$ to soluble fibrinogen labeled with Alexa Fluor 488 using FACS. BMMC bound soluble fibrinogen in an integrin $\alpha_{IIb}\beta_3$-dependent manner (Fig. 4*A*) when they were activated by HC IgE, IgE plus Ag, SCF, thrombin, or Mn^{2+}. It was previously shown that activated platelets bound to soluble fibrinogen and internalized it (24). To examine whether fibrinogen internalization occurs in mast cells as well, we analyzed the localization of soluble fibrinogen labeled with Alexa Fluor 488 in activated BMMC using a confocal microscope. Fibrinogen labeled with Alexa Fluor 488 was bound and internalized into the mast cells (Fig. 4*B*). This phenomenon was blocked by anti-integrin $\alpha_{IIb}\beta_3$ Ab, indicating that the activated mast cells can internalize fibrinogen via integrin $\alpha_{IIb}\beta_3$.

Human mast cells expressed integrin $\alpha_{IIb}\beta_3$ and mediated adhesion to fibrinogen

To confirm whether integrin $\alpha_{IIb}\beta_3$ is also expressed and is functional on human mast cells, human cord blood-derived mast cells (Fig. 5*A*) were generated as reported (42). Toluidine blue and tryptase staining confirmed that the purity of the mast cells exceeded 90%. FACS analysis showed that these cells expressed integrin $\alpha_{IIb}\beta_3$ (Fig. 5*B*). In addition, SCF- or $MnCl_2$-stimulated human cord blood-derived mast cells adhered to fibrinogen in an integrin $\alpha_{IIb}\beta_3$-dependent manner, as observed in the case of mouse BMMC and PMC (Fig. 5*C*). An experiment using the blocking Ab (2G12) confirmed that SCF-induced migration of the human mast cells was also enhanced via fibrinogen through integrin $\alpha_{IIb}\beta_3$ (data not shown)

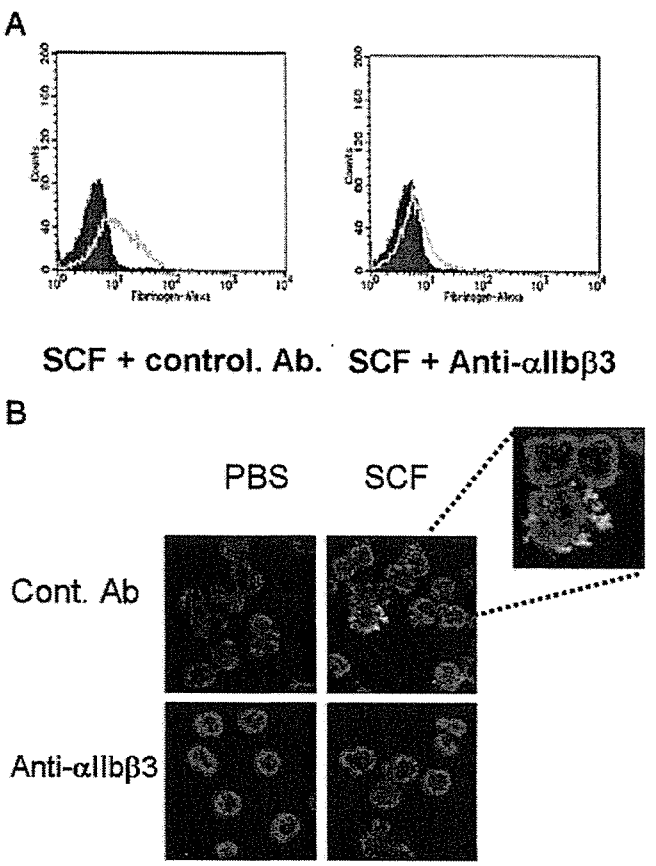

FIGURE 4. Uptake of soluble fibrinogen in SCF-stimulated BMMC in an integrin $\alpha_{IIb}\beta_3$-dependent manner. *A* and *B*, BMMC were incubated with 20 μg/ml soluble fibrinogen labeled with Alexa Fluor 488 (FB-Alexa) in the presence of 100 ng/ml SCF. *A*, In flow cytometry, SCF-stimulated BMMC bound soluble fibrinogen labeled with Alexa Fluor 488. *B*, In the analysis using a confocal laser microscope, the fibrinogen labeled with Alexa Fluor 488 was incorporated in the cells. This phenomenon was blocked by 10 μg/ml anti-integrin $\alpha_{IIb}\beta_3$ Ab.

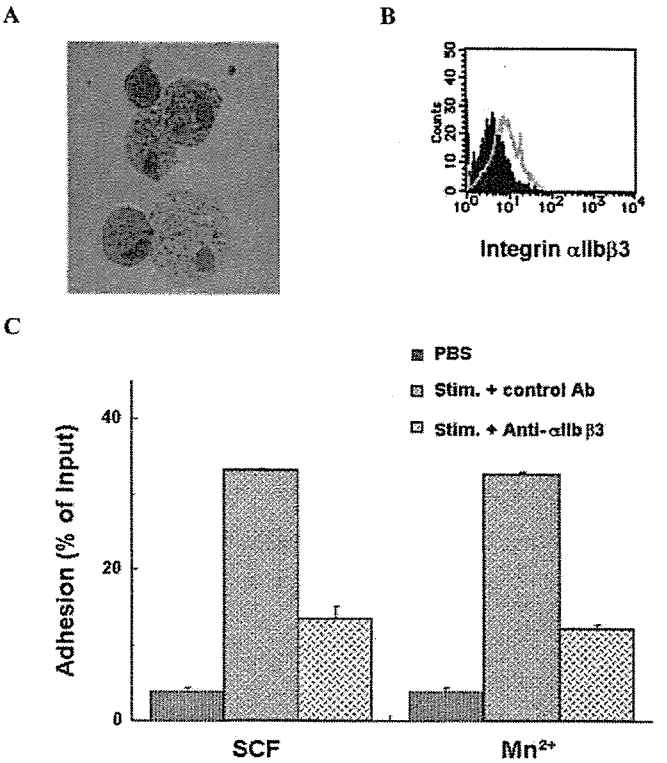

FIGURE 5. Human cord blood-derived mast cells express integrin $\alpha_{IIb}\beta_3$ and adhered to fibrinogen in an integrin $\alpha_{IIb}\beta_3$-dependent manner. *A*, Typical mast cells developed in the presence of SCF and IL-6 were stained with May-Grünwald Giemsa. *B*, Surface expression of integrin $\alpha_{IIb}\beta_3$ on human cord blood-derived mast cells was analyzed using flow cytometry. *C*, Adhesion of human cord blood-derived mast cells was induced by 100 ng/ml human SCF or 1 mM $MnCl_2$, and blocked by 60 μg/ml anti-integrin $\alpha_{IIb}\beta_3$ Ab (2G12).

Discussion

Expression of integrin $\alpha_{IIb}\beta_3$ on mast cells

Expression of integrin α_{IIb} was considered to be restricted to platelets/megakaryocytes. As recently reported, it turned out to be a marker for early hemapoietic progenitors as well (12–16)

In the present study, we demonstrate that integrin $\alpha_{IIb}\beta_3$ is expressed on several types of mouse mast cells, BMMC, PMC, skin- and lung-derived mast cells, and human cord blood-derived mast cells. There are two different types of mast cells, consisting of connective tissue and mucosal mast cells (1, 2). PMC and skin-derived mast cells represent connective tissue mast cells. BMMCs have characteristics similar to mucosal and connective tissue mast cells, depending on which factor their culture medium includes (IL-3 and IL-3 plus SCF, respectively). No remarkable change was observed in the expression level of integrin $\alpha_{IIb}\beta_3$ on BMMC under both culture conditions (data not shown). Thus, integrin $\alpha_{IIb}\beta_3$ is thought to be expressed on both types of matured mast cells. We observed a low level expression of integrin α_{IIb} only on a small population of bone marrow cells before the culture (data not

shown), but the percentage of integrin α_{IIb}^+/c-*kit*$^+$ cells and the expression level of integrin α_{IIb} gradually increased in culture along with the increase of FcϵRI$^+$/c-*kit*$^+$ cells, mast cell-lineage cells (44).

Next, we examined the expression of integrin α_{IIb} on basophils. However, it was difficult to confirm the expression on freshly isolated basophils from peripheral blood, because of the binding of platelet-derived microparticles, which transfer several kinds of platelet Ags like integrin α_{IIb} CD62 to other circulating blood cells (53). In contrast, BMMC FcϵRI$^+$/c-*kit*$^-$, mainly composed of basophils and basophil precursors (44, 54), express comparable levels of integrin α_{IIb}. As FcϵRI$^+$/c-*kit*$^-$ cells were generated after 10 days culture, the amount of platelet-derived microparticles binding to these cells was supposed to be negligible, indicating that bone marrow-derived basophils express integrin α_{IIb}. However it remains to be elucidated whether basophils circulating in peripheral blood express functional integrin α_{IIb}.

By contrast, no or negligible expression of integrin α_{IIb} was observed on splenic T cells, splenic B cells, granulocytes, macrophages, and dendritic cells. These results show that high expression of integrin α_{IIb} on mature cells is restricted to mast cells and bone marrow-derived basophils, except for platelets/megakaryocytes.

Although the precise mechanisms of the regulation of integrin α_{IIb} expression remain unknown, various transcription factors and their cofactors involved in megakaryocyte development, such as GATA-1, GATA-2, FOG-1, SCL, NF-E2, AML-1, Fli-1, Gfi-1b, MafB, Ets-1, and Ets-2 were implicated in the regulation of integrin α_{IIb} gene (55, 56). The expression of all these molecules was detected by RT-PCR both in megakaryocytes and mast cells, but not in mature cells of other lineages (data not shown). This result may illustrate, in part, the reason why integrin α_{IIb} is expressed on mast cells, which is in accordance with a previous report that mast cell progenitors and erythroid/megakaryocyte progenitors are closely related (57). Further investigation is underway to understand the regulatory mechanism of integrin expression.

Adhesion of mast cells to ECM proteins via integrin $\alpha_{IIb}\beta_3$

Various integrins are implicated in mast cell adhesion. Our evidence shows that integrin $\alpha_{IIb}\beta_3$ on mouse and human mast cells was functionally potent and mediated mast cell adhesion to fibrinogen and vWF. This finding was observed only when these mast cells were activated by some stimuli, including IgE plus Ag, HC IgE, and SCF. In contrast, the blockade of integrin $\alpha_{IIb}\beta_3$ by a specific Ab did not alter the adhesion of mast cells to VN and FN. This result is consistent with the previous result that the adhesion of mast cells to VN and FN is mainly mediated by integrins $\alpha_V\beta_3$ and β_1, respectively (5, 11, 28–31, 56).

Suehiro et al. (58) reported that purified integrin $\alpha_V\beta_3$ molecule had a higher affinity to VN than did integrin $\alpha_{IIb}\beta_3$. Kieffer et al. (59) showed that integrin $\alpha_{IIb}\beta_3$ expressed on a melanoma cell line and HEL selectively bound to fibrinogen, not to VN, whereas integrin $\alpha_V\beta_3$ on these cell lines mediated the adhesion to VN. Collectively, the integrin that is used in the interaction to VN, fibrinogen, and vWF could be determined by the different affinity and expression levels of integrins $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$.

Interestingly, bone marrow cells of integrin α_{IIb} knockout mice are reported to show decreased adhesive capacity to FN caused by impaired adhesive function of integrin $\alpha_4\beta_1$ and $\alpha_5\beta_1$, indicating cross-modulation of integrin α_{IIb} (15). This mode of modulation by integrin α_{IIb} was not detected in our study using specific blocking Abs, implying that the loss of the expression of integrin α_{IIb} and functional blockage of integrin $\alpha_{IIb}\beta_3$ might lead to distinct results.

Effects of attachment via integrin $\alpha_{IIb}\beta_3$ on mast cell functions

Attachment of mast cells to ECM proteins has been reported to enhance various mast cell functions. We demonstrate the evidence that the interaction between integrin $\alpha_{IIb}\beta_3$ and fibrinogen regulates mast cell functions in vivo. Recent studies have revealed that SCF and adhesion molecules like integrins are involved in mast cell-associated diseases. In addition, the role of fibrinogen and its degradation product, fibrin, in inflammatory reactions has been given focus. Importantly, SCF is known to critically define the accumulation of mast cells at the site of inflammation (1, 2, 37, 47, 60) including atherosclerotic plaques (9). Gurish et al. (37) showed that integrin $\alpha_4\beta_7$ is responsible for tissue-specific homing of mast cell progenitors to the small intestine during a helminth infection. Extravascular fibrinogen and fibrin, which also interact with integrin $\alpha_{IIb}\beta_3$, are abundant at the site of inflammation, such as arthritis (61), transplant rejection (62), bacterial infection (63–65), and atherosclerosis (66), where immune cells are recruited and activated (66). Collectively, the interaction of integrin $\alpha_{IIb}\beta_3$ with fibrinogen may be involved in mast cell-associated pathological conditions, especially where SCF is highly produced.

Another interesting aspect is that mast cells are known to express a profibrinolytic phenotype and contain fibrinolytic enzymes like tissue plasminogen activator and heparin in their granules (67). Moreover, mast cells are a major source of these enzymes (67). In accordance with these observations, mast cell deficiency leads to experimentally induced-thrombus formation and enhances thrombosis-associated mortality (67). It is tempting to assume that the adhesion of mast cell to fibrinogen augments such profibrinolytic phenotype by enhancing the effects of these fibrinolytic enzymes to lyse fibrinogen. The amount of fibrinogen was thought to be regulated by certain members of integrins using different mechanisms; human monocytes internalized and degraded fibrinogen, independently of plasmin activity via interaction with integrin $\alpha_M\beta_2$ (Mac-1) to clear fibrinogen and fibrin at injured or inflammatory sites (68). Activated platelets internalized fibrinogen via integrin $\alpha_{IIb}\beta_3$ to modulate the coagulation process (22–25). Interestingly, as we have presented in this paper, activated BMMC can also bind and internalize soluble fibrinogen in an integrin $\alpha_{IIb}\beta_3$-dependent manner (Fig. 4*B*). These results suggest that uptake of fibrinogen and fibrin via integrin $\alpha_{IIb}\beta_3$ leads to the clearance of fibrinogen and fibrin in inflammatory sites.

In conclusion, our novel findings show that integrin $\alpha_{IIb}\beta_3$ is expressed on mouse and human mast cells, and mediated adhesion to fibrinogen and vWF, resulting in the enhancement of mast cell functions in concert with SCF. A drug that regulates the function of integrin $\alpha_{IIb}\beta_3$ (33) may control the accumulation and activation of mast cells, leading to new therapeutic approaches forthcoming for mast cell-mediated diseases.

During our research, Berlanga et al. (69) reported the expression of integrin $\alpha_{IIb}\beta_3$ on BMMC. Unlike that report, we detected integrin $\alpha_{IIb}\beta_3$ both on BMMC and PMC. It might result from the difference in the affinity of the Abs against the integrin or in the mouse strains used. In fact, the expression levels of several integrins including integrin α_{IIb} on both types of mast cells were different between BALB/c and C57BL/6 mice (T. Oki, J. Kitaura, Y. Yamanishi, and T. Kitamura, unpublished observation). They suggested the in vivo function of integrin $\alpha_{IIb}\beta_3$ expressed on BMMC by showing that the adhesion to VN was increased in BMMC derived from the integrin α_{IIb} knockout mice. However this alteration was probably due to the compensatory enhanced expression of integrin α_V on BMMC of the integrin α_{IIb} knockout mice,

which we observed and later described; thus the function of integrin $\alpha_{IIb}\beta_3$ was not directly addressed. In contrast, we characterized functions of integrin $\alpha_{IIb}\beta_3$ on BMMC using a neutralizing Ab in multiple assays. In addition, Berlanga et al. (69) studied the mast cell functions in the absence of stimulation, whereas we investigated those in the presence and absence of the stimulation because the inside-out signaling is required for maximal adhesion of mast cells. Finally, using BMMC from integrin α_{IIb} knockout mice, a gift from Dr. J. Frampton (Institute of Biomedical Research, Birmingham University, Edgbaston, Birmingham, U.K.), we confirmed the following phenomena: the adhesion of these BMMC to VN was increased due to the enhanced expression of integrin $\alpha_V\beta_3$, whereas their adhesion to fibrinogen was significantly diminished because of the integrin α_{IIb} deficiency (data not shown). These results are in accordance with our results obtained from the study using blocking Abs, which indicate that the interaction of BMMC to fibrinogen and VN was mainly mediated by integrin $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$, respectively.

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Disclosures

The authors have no financial conflict of interest.

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