Mast cells have critical effector functions in various immune reactions. In allergic inflammation, mast cells interact with tissue-infiltrating eosinophils, forming a regulatory unit in the late and chronic phases of the allergic process. However, the pathways and molecules within this unit are still largely undefined. Here, we show that human mast cells and eosinophils express DNAX accessory molecule 1 (DNAM-1, CD226) and its ligand Nectin-2 (CD112). CD226 synergizes with FcεRI on mast cells, and its engagement augments degranulation through a pathway involving Fyn, linker of activation of T-cells, phospholipase Cγ2, and CD18. This pathway is subject to negative interference by inhibitory receptors and is completely inhibited by linking IgE with 1Rp60 (CD300a) using a bispecific antibody. Moreover, blocking CD112 expressed on eosinophils using neutralizing antibodies normalized the hyperactivity resulting from IgE-dependent activation of mast cells co-cultured with eosinophils. Our findings demonstrate a novel interface between these two effector cells, implicating relevance for in vivo allergic states. Moreover, costimulatory responses might be a critical component in allergic reactions and may therefore become novel targets for anti-allergic therapy.

During the past decade, mast cells have been recognized as critical immune effector cells. Solid evidence indicates their involvement in various settings extending far beyond their traditionally associated context of type I hypersensitivity reactions, such as innate immunity (1), autoimmunity (2), atherosclerosis (3), and more. Yet many aspects of their effector functions, even in the classical context of allergic reactions, are still unknown.

Until recently, mast cell involvement in the allergic process was confined to its early/acute phase, of which FcεRI-dependent activation forms the central trigger (4). The late/chronic phase, characterized by tissue inflammation and remodeling, was attributed solely to infiltrating leukocytes, notably to the eosinophils. However, several lines of evidence indicate that mast cells participate in modulation of the late/chronic phase mainly by their interactions with eosinophils and T lymphocytes. For example, mast cells induce T lymphocyte proliferation and enhance their activity through OX40/OX40L engagement (5). Reciprocally, intracellular adhesion molecule 1 (ICAM-1)/LFA-12 interactions costimulate mast cells upon contact with T lymphocytes (6). FcεRI-dependent activation of mast cells was shown to be enhanced by several receptors, including c-Kit (7), 4-1BB (8), CD28 (9), and CCR1 (10). Mast cell activity is also enhanced by multiple soluble factors as well. Moreover, it is likely to assume that positive signals, especially if operating via separate routes, will stack and synergize with each other.

Although mast cell-eosinophil interactions were shown to enhance cytotoxic functions in helminthic infections, the data regarding mast cell-eosinophil cross-talk in allergic settings are very scarce. Early observations have demonstrated that eosinophil major basic protein and eosinophil cationic protein activate mast cells and basophils to degranulate (11, 12). Eosinophils produce stem cell factor, potentially contributing to mast cell survival and activity (13). On the other hand, mast cells produce IL-3, IL-5, and GM-CSF, important cytokines regulating eosinophil recruitment, survival, and activity (14). Mast cell tryptase evokes eosinophil degranulation through the protease-activated receptor PAR2 (15). However, it is likely that information transfer between cells is mediated more by direct cell-cell interactions, being more efficient, accurate, and liable than paracrine communication systems.

In this study, we demonstrate that human mast cells express DNAX accessory molecule 1 (DNAM-1, CD226) and its ligands Nectin-2 (CD112) and the poliovirus receptor PVR (CD155). We show that CD226 augments FcεRI-mediated activation of mast cells by enhancing a signaling pathway dependent in part on Fyn, LAT, and PLCγ2 but not on Syk. Moreover, this pathway involves association of CD226 with integrins, and preferentially to CD18. We also demonstrate that this pathway is prone to inhibition by inhibitory receptors. Finally, we show that in the presence of eosinophils, mast cell FcεRI-mediated activation is augmented and that this effect is mediated at least partially by a CD226/CD112 interaction. In conclusion, these findings demonstrate a novel interface between mast cells and eosinophils and suggest that CD226 and CD112 participate in modulation of the allergic response.

The abbreviations used are: LFA-1, leukocyte function-associated antigen 1; DNAM-1, DNAX accessory molecule 1; PVR, poliovirus receptor; ICAM-1, intracellular adhesion molecule 1; LAT, linker for activation of T cells; ERK, extracellular-signal regulated kinase; MAPK, mitogen-activated protein kinase; PLC, phospholipase C; HMC-1, human mast cell leukemia 1; FACS, fluorescence-activated cell sorter; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

8 This work was supported by grants from the Aimwell Charitable Trust (UK), the Israeli Science Foundation (Israel) and a grant from the Italian Ministry of Foreign Affairs for joint research with Israel (Italy). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
9 Affiliated with the David R. Bloom Center of Pharmacy at the Hebrew University of Jerusalem. To whom correspondence should be addressed. Tel: 972-2-6757512; Fax: 972-2-6758144; E-mail: ffs@cc.huji.ac.il.

2 The abbreviations used are: LFA-1, leukocyte function-associated antigen 1; DNAM-1, DNAX accessory molecule 1; PVR, poliovirus receptor; ICAM-1, intracellular adhesion molecule 1; LAT, linker for activation of T cells; ERK, extracellular-signal regulated kinase; MAPK, mitogen-activated protein kinase; PLC, phospholipase C; HMC-1, human mast cell leukemia 1; FACS, fluorescence-activated cell sorter; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Mast Cell Costimulation by CD226/CD112 (DNAM-1/Nectin-2)
A NOVEL INTERFACE IN THE ALLERGIC PROCESS

Received for publication, March 13, 2006, and in revised form, June 13, 2006. Published, JBC Papers in Press, July 10, 2006, DOI 10.1074/jbc.M602359200

Ido Bachelet, Ariel Munitz, David Mankutad, and Francesca Levi-Schaffer

From the Department of Pharmacology, The School of Pharmacy, The Faculty of Medicine, The Hebrew University of Jerusalem and the Department of Obstetrics and Gynecology, Hadassah University Hospital, Jerusalem 91120, Israel.
EXPERIMENTAL PROCEDURES

Antibodies and Reagents—All the cell culture media, reagents, and buffers were purchased from Biological Industries, Beit Haemek, Israel. Stem cell factor is a kind gift from Amgen, Inc. (Thousand Oaks, CA). IL-6 was purchased from Peprotech (Rocky Hill, NJ). The following mouse monoclonal antibodies were prepared as described (16, 17) and used: F22 (anti-DNAM-1/CD226, IgG1), F5 (anti-DNAM-1/CD226, IgM), L14 (anti-Nectin-2/CD112, IgG2a), L95 (anti-PRV/CD155, IgG1), E59 (anti-CD300a, IgG1), P192 (anti-CD300a, IgG2a). The following antibodies and reagents were purchased and used: rat anti-mouse CD300a (clones (NKRL1-172206.111, -172219.111, -172224.111, -172238.111)) from R&D Systems (Minneapolis, MN); mouse anti-human IgE (clone GE-1) from Sigma; anti-human trypsin (clone AA1) and isotype control (IgG1 and IgG2A) antibodies from Dako (Glostrup, Denmark); sheep anti-mouse F(ab\’)2 from ICN Biomedicals (Aurora, OH); human myeloma IgE/\kappa from Calbiochem-Merck (Schwalbach, Germany); polyclonal anti-human phosphotyrosine, phosphoserine, Fyn, CD18 and CD29 from Santa Cruz Biotechnology (Santa Cruz, CA); rabbit anti-human phospho-syk, phospho-LAT, phospho-ERK, phospho-PLC\gamma 1, and phospho-PLC\gamma 2 from Cell Signaling (Beverly, MA); horseradish peroxidase-conjugated anti-rabbit and anti-mouse, fluorescein isothiocyanate-, CY5-, and phycoerythrin-conjugated secondary antibodies from Jackson Laboratories (West Grove, PA); chromogenic substrates from Sigma; Calcium Green-1AM from Molecular Probes (Eugene, OR); protein G from Pierce; and Ficol-paque from Amersham Biosciences (Uppsala, Sweden). All other reagents were from Sigma, unless otherwise stated, and were of best chemical grade available. FcR blocking reagent 2.4G2 was purchased from BD Biosciences.

Cell Culture—Human cord blood-derived mast cells were obtained and cultured as described (16). Briefly, mononuclear progenitors were isolated from cord blood by Ficoll gradient and cultured in α-MEM/10% fetal calf serum in the presence of stem cell factor, IL-6, and prostaglandin E2 for at least 7–8 weeks, with weekly refreshment of the culture medium. At weeks 7–8, mature mast cells were identified using toluidine blue and tryptase staining by FACS. Cells were used upon reaching >95% pure cultures. Eosinophils were purified from peripheral blood of untreated mildly atopic individuals (blood eosinophil levels >5%) as described (18). Briefly, eosinophils were isolated by a serial procedure of dextran sedimentation, Ficoll gradient, and magnetic cell sorting using anti-CD3/anti-CD16 beads and brought to >98% purity in RPMI 1640/10% fetal calf serum, assessed by Kimura’s staining.

HMC-1 cell line (a kind gift from Dr. J. Butterfield, Mayo Clinic, Rochester, MN) is maintained routinely in our laboratory and was cultured in Iscove’s modified Dulbecco’s medium/10% fetal calf serum with refreshment of the medium upon reaching a cell density of 5 × 10^6 cells/ml. Cord blood and peripheral blood were obtained upon signed consent of the donors, and the entire experimental plan was reviewed and approved by the Hadassah Hospital Helsinki Committee for Human Experimentation.

Mast Cell Costimulation by CD226/CD112

Activation System and Mediator Assays—For mediator assays, cells were activated in Immunolon-2HB 96-well plates (ThermoLabsystems, MA). The plates were coated with sheep anti-mouse F(ab\’)2 (25 μg/ml) followed by anti-CD226 (clone F5), anti-CD112, anti-CD155, or isotype control (10 μg/ml, 2 h at 37 °C). Mast cells were sensitized with human myeloma IgE (5 μg/ml, 2 h at 37 °C) 5 days prior to activation. On activation, cell were washed with Tyrode’s buffer (137 mM NaCl, 12 mM NaHCO3, 5.5 mM L-glucose, 2 mM KCl, 0.3 mM Na2HPO4, 1.8 mM CaCl2, 0.9 mM MgCl2), added to the plate on ice simultaneously with mouse anti-human IgE antibody (5 μg/ml), and the plate was incubated at 37 °C for 30 min. Activation was stopped by removing supernatants from cells by centrifugation and freezing of both fractions for analysis. Tryptase was analyzed by a chromogenic assay as described (16). IL-4 was analyzed by a commercial Duoset enzyme-linked immunosorbent assay kit (Diaclone, Besançon, France). Prostaglandin D2 was analyzed by a commercial enzymatic immunoassay kit (Cayman, Ann Arbor, MI).

For intracellular flow cytometry, antibody incubations (specific and sheep anti-mouse antibody) and activation were performed with cells in suspension (30 min on ice for each antibody incubation). Cells were fixed at the stated time points (see below). For calcium mobilization, antibody incubations (specific and sheep anti-mouse antibody) were performed with cells in suspension (30 min on ice for each incubation), and cells were loaded with Calcium Green-1AM prior to activation (see below).

Flow Cytometry—For surface cytometry, cells were washed once with cold HBA buffer (0.1% w/v bovine serum albumin, 0.05% w/v NaN3 in Hanks’ solution), and both primary and secondary antibody incubations were performed in HBA buffer (30 min on ice with two washes after each incubation). For intracellular cytometry, cells were fixed (2% v/v formaldehyde in Hanks’ solution, 10 min on ice) at the stated time points (see Results), permeabilized and blocked (5% w/v bovine serum albumin, 1% v/v human serum, 0.1% w/v saponin, 10 mM HEPES, 0.05% w/v NaN3 in Hanks’ solution, 15 min on ice), and transferred to incubation buffer (0.1% v/v bovine serum albumin, 0.1% w/v saponin, 10 mM HEPES, 0.05% w/v NaN3 in Hanks’ solution). Primary and secondary antibody incubations were performed in incubation buffer (30 min on ice with two washes after each incubation). Data were acquired in a BD Biosciences FACScalibur and analyzed using a CellQuest software on a Mac-based work station.

Intracellular Ca2+ Mobilization—After antibody incubations, cells were loaded with Calcium Green-1AM (5 μM, 45 min, 37 °C) in α-MEM (2% v/v fetal calf serum), washed, and resuspended in 300 μl of Tyrode’s gelatin buffer (Tyrode’s buffer containing 0.1% w/v gelatin) prewarmed to 37 °C. The cells were allowed to flow freely in the cytometer for 30 s. At this time, mouse anti-human IgE antibody (5 μg/ml) was added. Changes in the FL-1 geo mean were recorded for a total of 3 min.

Western Blot and Immunoprecipitation—After antibody incubations and activation, cells (10^6/group) were lysed using M-PER lysis buffer (Pierce) supplemented with protease inhibitor mix (Sigma). Cell lysates were run on SDS-PAGE, trans-
Mast Cell Costimulation by CD226/CD112

ferred to polyvinylidene difluoride membrane, and blotted versus specific proteins (see Results). Immunoprecipitations were performed with a commercial kit (Sieze™ Classic Mammalian Kit, Pierce) according to the manufacturer’s instructions, using $7 \times 10^6$ cells/group. The antibodies used to precipitate CD226 were clone F22, which do not interfere with CD226 binding by clone F5 antibodies (see above). In some cases, cells were treated with $4 \text{ mM Na}_3\text{VO}_4$ (Sigma) for 10 min before lysis. Blotted membranes were developed using ECL Plus (Amersham Biosciences) according to the manufacturer’s instructions.

Inhibition Assay—The bispecific antibody linking CD300a to IgE (IE14) was generated as described elsewhere (19). IE14 was used to treat mast cells activated with IgE and anti-CD226 as described above.

Mast Cell-Eosinophil Co-culture—Freshly isolated eosinophils were blocked with 10% v/v human serum in RPMI 1640 (10 min on ice) followed by 2.4G2 Fc receptor blocking reagent (10 min on ice), incubated with anti-CD226, anti-CD112, anti-CD155, or isotype control (10 $\mu$g/ml, 10 min on ice), and washed. Other groups were further treated with GM-CSF (50 ng/ml, 20 min at 37 °C) and washed extensively. Eosinophils were then added to mast cells that were treated as described above (activation system) to the plate simultaneously with anti-human IgE antibody. Activation was terminated, and mediators were analyzed as described above.

Statistical Analysis—Mediators were measured in triplicates and in at least three different sets of experiments or donor batches. Data are always the mean ± S.D. and were analyzed by analysis of variance followed by paired Student’s $t$ test assuming equal variances.

RESULTS

CD226 (DNAM-1), CD112 (Nectin-2), and CD155 (PVR) have been shown to mediate and modulate lymphocyte costimulation and effector functions such as tumor cell lysis (20, 21). Moreover, a recent study reported that these receptors are expressed on dendritic cells, thus having important consequences on dendritic cell-lymphocyte interactions (22).

We hypothesized that these molecules may modulate mast cell effector functions in allergic inflammation as well, with a strong emphasis on the manner by which these molecules can mediate information flow between mast cells and eosinophils. We were first set, therefore, to investigate whether CD226, CD112, and CD155 are expressed on mast cells and eosinophils. For this, human mast cells and eosinophils were screened using monoclonal antibodies recognizing CD226, CD112, and CD155. Flow cytometric analysis revealed that mast cells expressed high levels of CD226 and CD112 and negligible levels of CD155. Eosinophils expressed only high levels of CD112 and low levels of CD226 and CD155. We next examined the expression of these receptors on HMC-1 cells in comparison with their expression on primary mast cells. HMC-1 expressed high levels of all three receptors, including CD155, as opposed to the pattern observed on mast cells or eosinophils (Fig. 1).

Once expression of these receptors on mast cells was established, we wished to discern whether they are capable of modulating signal transduction and activation in these cells. For this purpose, mast cells were activated by FcεRI-IgE cross-linking, simultaneously with antibody-mediated engagement of CD226, CD112, and CD155. We have also activated cells with combinatorial linking (CD226+CD112, CD226+CD155, and CD112+CD155).

Upon engagement of CD226, a substantial increase in FcεRI-induced release of tryptase and IL-4 (Fig. 2A and data not shown, respectively) and in eicosanoid synthesis (Fig. 2B) was observed. This phenomenon was not shared by CD112 and CD155 or by the isotype control antibodies. Furthermore, the combinatorially linked cells displayed augmented degranulation only when CD226 was engaged.

The mechanism by which CD226 operates in lymphocytes has been studied (23, 24). However, it is still not known whether this mechanism is common regarding all the receptors that collaborate with CD226. Furthermore, mechanisms of mast cell costimulation have been reported that involve integrins, tumor necrosis factor superfamily receptors, chemokine receptors, and others. Thus, no particular hypothesis directed this stage of the study, and we adopted a screening approach of several signaling molecules critical within the mast cell activation cascade.

We have examined the phosphorylation states of syk, LAT, ERK (p44/42 MAPK), PLCγ1, and PLCγ2 in FcεRI-activated/CD226-engaged mast cells. At $t = 15$ min, CD226 augmented the phosphorylation of LAT and PLCγ2 but not of PLCγ1 (Fig. 3A). ERK phosphorylation was increased in CD226-engaged cells at $t = 30$ min. Syk phosphorylation levels remained unchanged in all experimental groups.

Degranulation is ultimately induced by a cytosolic calcium influx. We have therefore examined the effect of CD226 on the mobilization of intracellular calcium. We found that CD226 induced a significantly increased calcium influx with an immediate onset (Fig. 3B).

As a substrate of protein kinase activity, CD226 has been shown to possess a serine residue mediating its recruitment into lipid rafts and a tyrosine residue mediating its costimulatory activity (24). Our next aim was therefore to investigate whether CD226 on mast cells undergoes the same changes as a
part of its signaling mechanism. We have immunoprecipitated CD226 from FcεRI/RI-activated/CD226-engaged mast cells and used Western blot to detect phosphorylated serine. Indeed, as shown (Fig. 4), CD226 is serine-phosphorylated upon its engagement.

To identify the signaling events induced by CD226 in activated mast cells, we have screened for proteins with increased tyrosine phosphorylation level that occur upon CD226 engagement. Immunoprecipitation versus CD226 and Western blot analysis showed that in FcεRI/RI-activated/CD226-engaged cells, tyrosine-phosphorylated proteins that bind to CD226 include the following species (in kDa): ~150, ~125, ~65, ~55, and ~45 (Fig. 5A). The p65 species correlate with CD226 itself since Syk signaling was not increased following CD226 engagement. On the other hand, LAT phosphorylation was shown to be augmented by CD226, and therefore, it is most likely to correlate to the p45 species.

Based on reports concerning the mechanism of CD226 activity in other cells, we hypothesized that the p55 and p125/p150 species correlate to Fyn and β integrins, respectively, both of which have been shown to associate physically with CD226 in
lymphocytes. To test this hypothesis, we first blotted immunoprecipitated CD226 versus Fyn and found that Fyn is physically associated with CD226 already upon FcεRI/H9280RI-induced activation, but this association is substantially increased upon CD226 engagement (Fig. 5B).

Second, we have blotted CD226 versus CD29 and CD18 and observed that CD226 was strongly associated with CD18, but not with CD29, upon its engagement, although a lesser extent of association was also evident upon FcεRI-induced activation (Fig. 5C). To discern whether this is due to inability of CD29 to bind to CD226 in mast cells, we have precipitated CD29 and CD18 from Na3VO4-treated cells and blotted them versus CD226. Indeed, CD226 associated preferentially to CD18, although a weak CD29 association was also observed (Fig. 5D). This figure is representative of three experiments.

Mast Cell Costimulation by CD226/CD112
In this study, we report that CD226 (DNAM-1) functions as a costimulatory receptor on human mast cells. Moreover, we report that CD226 is engaged by CD112 on human eosinophils to elicit its costimulatory effect. Based on these two lines of evidence, we present a novel interface between human mast cells and eosinophils that may constitute an important component in allergic disorders.

Expression analysis revealed that mast cells express high levels of CD226 and CD112, whereas expressing very low levels of CD155. However, only CD112 was expressed in high levels on eosinophils. This observation may provide an important hint as to the structure of the network between mast cells and eosinophils.

Strikingly, leukemic HMC-1 cells expressed very high levels of CD155, supporting previously described observations (22, 28). CD155 can also be down-regulated, for example by cytomegalovirus UL141 (29). This plasticity raised the question regarding the capability of various mediators to modulate CD226, CD112, and CD155 on mast cells. However, no mediator we have tested so far could induce a change in any of these molecules on human mast cells (data not shown).

It has been recently shown that CD226 augments the activation induced by several receptors containing an ITAM, e.g. CD16 and Nkp46, as well as by receptors containing an ITSM, e.g. 2B4 (CD244) (30). We therefore hypothesized that FcεRI-mediated signaling will also be enhanced by cross-linking with CD226. Indeed, we have observed a costimulatory effect of CD226 on FcεRI, a multiple ITAM-bearing receptor. This phenomenon could mean that mast cells in vivo require CD226 to develop a full intensity allergic response. Conversely, CD226 engagement alone was not sufficient to induce mast cell activation, suggesting only a modulatory role on mast cells.

CD226 exerts its effect through a distinct signaling pathway. Analysis of signaling molecule phosphorylation indicated that Syk is not included in this pathway. On the other hand, LAT and PLCγ2 were substantially more phosphorylated upon CD226 engagement. This is also in support of previously described observations (7, 8, 31, 32). Other kinases, such as Btk (8, 9), could also be involved in this pathway. Intriguingly, maximal phosphorylation of CD226 was observed at 5 min, whereas LAT and ERK phosphorylation peaked at 15 min. This accounts for the fact that LAT and ERK are both downstream and independent of CD226, and although these signals propagate (15 min), CD226 rapidly reverts back to its standby state.

CD226 on T cells was shown to associate with LFA-1, a complex involving CD18 (23). In addition, LFA-1 mediates costimulation of mast cells by T cells (6). We therefore anticipated a β2-associated mechanism. Indeed, CD226 on mast cells bound preferentially to CD18, although it displayed an ability to bind to CD29 as well. This versatility could indicate that no limitations exist for synergy between activating pathways, in the sense that different cascades will not have to compete over a limited pool of common signaling molecules.

Based on our hypothesis that CD226-induced costimulation might contribute to a fully developed allergic responses in vivo, we view CD226 as a valuable target in allergic diseases. As we have shown previously, mast cells express CD300a, a potent inhibitory receptor capable of blocking FcεRI-induced degran-
Mast Cell Costimulation by CD226/CD112

ulation. Our findings here demonstrate that CD226-induced signaling is prone to blockade by ITIM-bearing inhibitory receptors, and therefore, this could be considered in the future as a new approach in allergy therapy as well (33).

A central question raised by our study is whether eosinophils communicate with mast cells through costimulatory receptor/ligand interactions. This question is crucial in allergic diseases since mast cells and eosinophils together are the key effector cells in these settings. Yet the data regarding their interactions, in any setting, are very scarce.

Eosinophils and mast cells may mutually activate and enhance each other’s functions by a variety of signals, most of them secreted. We initiated a large scale screening of surface molecules to identify candidate receptor/ligand pairs that enhance each other’s functions by a variety of signals, most of ligand interactions. This question is crucial in allergic diseases as a new approach in allergy therapy as well (33).

In conclusion, we show here a novel interface between mast cells and eosinophils, with important possible consequences on chronic allergic processes and other diseases on which these two cells are associated. As mentioned, blocking this interface may have a critical value in future therapy of allergic diseases.

Acknowledgments—We thank Prof. A. Moretta and L. Moretta for providing DNA-M-1, Nectin-2, and PVR antibodies used in this study, Prof. Y. A. Mekori for helpful discussions, and all the members of the F. L. S. laboratory.

REFERENCES

1. Malaviya, R., Ikeda, T., Ross, E., and Abraham, S. N. (1996) Nature 381, 77–80
2. Secor, V. H., Secor, W. E., Gutekunst, C. A., and Brown, M. A. (2000) J. Exp. Med. 191, 813–822
3. Lappalainen, H., Laine, P., Pentikainen, M. O., Sajantila, A., and Kovanen, P. T. (2004) Arterioscler. Thromb. Vasc. Biol. 24, 1880–1885
4. Galli, S. J., Kalesnikoff, J., Grimaldi, J., Ayala, J., Miller, A. M., Williams, C. M., and Tsai, M. (2005) Annu. Rev. Immunol. 23, 749–786
5. Kashikawa, J., Yoko, H., Saito, H., and Okumura, Y. (2004) J. Immunol. 173, 5247–5257
6. Inamura, N., Mekori, Y. A., Bhattacharyya, S. P., Blanchine, P. J., and Metcalfe, D. D. (1998) J. Immunol. 160, 4026–4033
7. Hundley, T. R., Gielaff, A. M., Tkaczuk, C., Andrade, M. V., Metcalfe, D. D., and Beaven, M. A. (2004) Blood 104, 2410–2417
8. Nishimoto, H., Lee, S. W., Hong, H., Potter, K. G., Maeda-Yamamoto, M., Kinoshita, T., Kawakami, Y., Mittler, R. S., Kwon, B. S., Ware, C. F., Croft, M., and Kawakami, T. (2005) Blood 106, 4241–4248
9. Tashiro, M., Kawakami, Y., Abe, R., Han, W., Hata, D., Sugie, K., Yao, L., and Kawakami, T. (1997) J. Immunol. 158, 2382–2389
10. Toda, M., Dawson, M., Nakamura, T., Munro, P. M., Richardson, R. M., Bailly, M., and Ono, S. J. (2004) J. Biol. Chem. 279, 48434–48448
11. Zheutlin, T. M., Ackerman, S. J., Geilch, G. J., and Thomas, L. L. (1984) J. Immunol. 133, 2180–2185
12. O'Donnell, M. C., Ackerman, S. J., Geilch, G. J., and Thomas, L. L. (1983) J. Exp. Med. 157, 1981–1991
13. Hartman, M., Piliponsky, A. M., Temkin, V., and Levi-Schaffer, F. (2001) Blood 97, 1086–1091
14. Osler, C., Milon, G., Braquet, P., Mencia-Huerta, J. M., and David, B. (1996) Cell. Immunol. 167, 205–215
15. Temkin, V., Kantor, B., Weg, V., Hartman, M., and Levi-Schaffer, F. (2002) J. Immunol. 169, 2662–2669
16. Bachelet, I., Munitz, A., Moretta, A., Moretta, L., and Levi-Schaffer, F. (2005) J. Immunol. 175, 7989–7995
17. Bottino, C., Castriconi, R., Pende, D., Rivera, P., Nanni, M., Carnemolla, B., Cantonin, C., Grassi, J., Marcenaro, S., Reymond, N., Vitale, M., Moretta, L., Lopez, M., and Moretta, A. (2003) J. Exp. Med. 195, 557–567
18. Munitz, A., Bachelet, I., Elshar, R., Moretta, A., Moretta, L., and Levi-Schaffer, F. (2006) Blood 107, 1996–2003
19. Bachelet, I., Munitz, A., and Levi-Schaffer, F. (2006) J. Allergy Clin. Immunol. 117, 1314–1320
20. Shibuya, A., Campbell, D., Hannum, C., Yssell, H., Franz-Bacon, K., McClaunahan, T., Kitamura, T., Nicholl, J., Sutherland, G. R., Lanier, L. L., and Phillips, J. H. (1996) Immunity 4, 573–581
21. Shibuya, K., Shirakawa, K., Kameyama, T., Honda, S., Tahara-Hanaoka, S., Miyamoto, A., Onodera, M., Sumida, T., Nakauhi, H., Miyoshi, H., and Shibuya, A. (2003) J. Exp. Med. 198, 1829–1839
22. Pende, D., Castriconi, R., Romagnani, P., Spaggiari, G. M., Marcenaro, S., Dondero, A., Lazzery, E., Lasagna, L., Martini, S., Rivera, P., Capobianco, A., Moretta, L., Moretta, A., and Bottino, C. (2006) Blood 107, 2030–2036
23. Shibuya, K., Lanier, L. L., Phillips, J. H., Ochs, H. D., Shimizu, K., Nakayama, E., Nakauhi, H., and Shibuya, A. (1999) Immunity 11, 615–623
24. Shirakawa, J., Shibuya, K., and Shibuya, A. (2005) Int. Immunol. 17, 217–223
25. Kay, A. B. (2001) N. Engl. J. Med. 344, 30–37
26. Tahara-Hanaoka, S., Shibuya, K., Onoda, Y., Zhang, H., Yamazaki, S., Miyamoto, A., Honda, S., Lanier, L. L., and Shibuya, A. (2004) Int. Immunol. 16, 533–538
27. Tahara-Hanaoka, S., Shibuya, K., Kai, H., Miyamoto, A., Morikawa, Y., Ohkouchi, K., Honda, S., and Shibuya, A. (2006) Blood 107, 1491–1496
28. Masson, D., Jarry, A., Baury, B., Blanchardie, P., Laboisse, C., Lustenberger, P., and Denis, M. G. (2001) Gut 49, 236–240
29. Tomasec, P., Wang, E. C., Davison, A. J., Vajteske, B., Armstrong, M., Griffin, C., McSharry, B. P., Morris, R. J., Llewellyn-Lacey, S., Rickards, C., Rickards, C., and Wilkinson, G. W. (2005) Nat. Immunol. 6, 181–188
30. Bryceson, T. Y., March, M. E., Luongen, H. G., and Long, E. O. (2006) Blood 107, 159–166
31. Astier, A., Tresco-Biennet, M. C., Azoroc, O., Lamoulle, B., and Rabourdin-Combe, C. (2000) J. Immunol. 164, 6091–6095
32. Leyton, L., Quest, A. F., and Bron, C. (1999) Mol. Immunol. 36, 755–768
33. Kallinich, T., Beier, K. C., Gelfand, E. W., Kroczek, R. A., and Hamelmann, E. (2005) Clin. Exp. Allergy 35, 1521–1534
34. Munitz, A., Bachelet, I., Frangkel, S., Katz, G., Mandelboim, O., Simon, H. U., Moretta, L., Colonna, M., and Levi-Schaffer, F. (2005) J. Immunol. 174, 110–118
35. Malaviya, R., Gao, Z., Thankavel, K., van der Merwe, P. A., and Abraham, S. N. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 8110–8115
36. Shin, J. S., Gao, Z., and Abraham, S. N. (2000) Science 289, 785–788
37. Shin, J. S., and Abraham, S. N. (2001) FEMS Microbiol. Lett. 197, 131–138