発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kato K, Satoh T, Tanaka T, Ueda N, Yokozeki H.	Systemic nickel allergy presenting as papuloerythroderma-loke eruptions.	Acta Derm Venereol	90	655-6	2010
Kira M, Katayama I.	Superimposed linear psoriasis.	J Dermatol.	37(12)	1063-5	2010
Matsui S, Kitaba S, Itoi S, Kijima A, Murota H, Tani M, Katayama I.	A case of disseminated DLE complicated by atopic dermatitis and Sjögren's syndrome: link between hypohidrosis and skin manifestations.	Mod Rheumatol.	21(1)	101-5	2010
Matsushima Y, Kikkawa Y, Takada T, Matsuoka K, Seki Y, Yoshida H, Minegishi Y, Karasuyama H, and Yonekawa H	An atopic dermatitis-like skin disease with hyper-IgEemia develops in mice carrying a spontaneous recessive point mutation in the Traf3ip2 (Act1/CIKS) gene.	J. Immunol.	185	2340-49	2010
Moniaga CS, Egawa G, Kawasaki H, Hara-Chikuma M, Honda T, Tanizaki H, Nakajima S, Otsuka A, Matsuoka H, Kubo A, Sakabe J, Tokura Y, Miyachi Y, Amagai M, Kabashima K.	Flaky tail mouse denotes human atopic dermatitis in the steady state and by topical application with Dermatophagoides pteronyssinus extract.	Am J Pathol.	176	2385-93	2010
Mori T, Ishida K, Mukumoto S, Yamada Y, Imokawa G, Kabashima K, Kobayashi M, Bito T, Nakamura M, Ogasawara K, Tokura Y	Comparison of skin barrier function and sensory nerve electric current perception threshold between IgE-high extrinsic and IgE-normal intrinsic types of atopic dermatitis.	Br J Dermatol.	162	83-90	2010
Murota H, El-latif MA, Tamura T, Amano T, Katayama I.	Olopatadine hydrochloride improves dermatitis score and inhibits scratch behavior in NC/Ngamice.	Int Arch Allergy Immunol.	153(2)	121-32	2010
Murota H, Kitaba S, Tani M, Wataya-Kaneda M, Azukizawa H, Tanemura A, Umegaki N, Terao M, Kotobuki Y, Katayama I.	Impact of sedative and non-sedative antihistamines on the impaired productivity and quality of life in patients with pruritic skin diseases.	Allergol Int.	59(4)	345-54	2010
Murota H, Kitaba S, Tani M, Wataya-Kaneda M, Katayama I.	Effects of nonsedative antihistamines on productivity of patients with pruritic skin diseases.	Allergy	65(7)	929-30	2010
Murota H, Takahashi A, Nishioka M, Matsui S, Terao M, Kitaba S, Katayama I.	Showering reduces atopic dermatitis in elementary school students.	Eur JDermatol.	20(3)	410-1	2010
Nakajima S, Honda T, Sakata D, Egawa G, Tanizaki H, Otsuka A, Moniaga CS, Watanabe T, Miyachi Y, Narumiya S, Kabashima K.	Prostaglandin I2-IP signaling promotes Th1 differentiation in a mouse model of contact hypersensitivity.	J Immunol.	184	5595-603	2010
Nishimura Y, Yamaguchi Y, Tomita Y, Hamada K, Maeda A, Morita A, Katayama I.	Epithelioid sarcoma on the foot masquerading as an intractable wound for > 18 years.	Clin Exp Dermatol.	35(3)	263-8	2010

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nishioka M, Tanemura A, Yamanaka T, Tani M, Miura H, Asakura M, Tamai N, Katayama I.	Pilomatrix carcinoma arising from pilomatricoma after 10-year senescent period: Immunohistochemical analysis.	J Dermatol.	37(8)	735-9	2010
Satoh T, Shimura C, Miyagishi C, Yokozeki H.	Indomethacin-induced reduction of CRTH2 in eosinophilic pustular folliculitis (Ofuji's disease): A proposed mechanism of action.	Acta Derm Venereol.	90	18-22	2010
Shima Y, Kuwahara Y, Murota H, Kitaba S, Kawai M, Hirano T, Arimitsu J, Narazaki M, Hagihara K, Ogata A, Katayama I, Kawase I, Kishimoto T, Tanaka T.	The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab.	Rheumatology (Oxford).	49(12)	2408-12	2010
Shimura C, Satoh T, Igawa K, Aritake K, Urade Y, Nakamura M, Yokozeki H.	Dendritic cells express hematopoietic prostaglandin D synthase and function as a source of prostaglandin D2 in the skin.	Am J Pathol	176(1)	227-37	2010
Sugita K, Kabashima K, Sakabe J, Yoshiki R, Tanizaki H, Tokura Y.	FTY720 regulates bone marrow egress of eosinophils and modulates late-phase skin reaction in mice.	Am J Pathol.	177	1881-7	2010
Takahashi Y, Murota H, Tarutani M, Sano S, Okinaga T, Tominaga K, Yano T, Katayama I.	A case of juvenile dermatomyositis manifesting inflammatory epidermal nevus-like skin lesions: unrecognized cutaneous manifestation of blaschkitis?	Allergol Int.	59(4)	425-8	2010
Takamatsu H, Takegahara N, Nakagawa Y, Tomura M, Taniguchi M, Friedel RH, Rayburn H, Tessier-Lavigne M, Yoshida Y, Okuno T, Mizui M, Kang S, Nojima S, Tsujimura T, Nakatsuji Y, Katayama I, Toyofuku T, Kikutani H, Kumanogoh A.	Semaphorins guide the entry of dendritic cells into the lymphatics by activating myosin II.	Nat Immunol.	11(7)	594-600	2010
Tanizaki H, Egawa G, Inaba K, Honda T, Nakajima S, Moniaga CS, Otsuka A, Ishizaki T, Tomura M, Watanabe T, Miyachi Y, Narumiya S, Okada T, Kabashima K.	Rho-mDia1 pathway is required for adhesion, migration, and T-cell stimulation in dendritic cells.	Blood	116	5875-84	2010
Terao M, Sakai N, Higashiyama S, Kotobuki Y, Tanemura A, Wataya-Kaneda M, Yutsudo M, Ozono K, Katayama I.	Cutaneous symptoms in a patient with cardiofaciocutaneous syndrome and increased ERK phosphorylation in skin fibroblasts.	Br J Dermatol.	163(4)	881-4	2010
Tokura Y.	Extrinsic and intrinsic types of atopic dermatitis.	J Dermatol Sci.	58	1-7	2010

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tomura M, Honda T, Tanizaki H, Otsuka A, Egawa G, Tokura Y, Waldmann H, Hori S, Cyster JG, Watanabe T, Miyachi Y, Kanagawa O, Kabashima K.	Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice.	J Clin Invest.	120	883-93	2010
Torrero M.N, Hubner M.P, Larson D, Karasuyama H, and Mitre E.	Basophils amplify type 2 immune responses, but do not serve a protective role, during chronic infection of mice with the filarial nematode Litomosoides sigmodontis.	J. Immunol.	185	7426-34	2010
Wada T, Ishiwata K, Koseki H, Ishikura T, Ugajin T, Ohnuma N, Obata K, Ishikawa R, Yoshikawa S, Mukai K, Kawano Y, Mineg, Yokishi Yokozeki H, Watanabe N, Karasuyama H.	Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks	J Clin Invest.	120(8)	2867-75	2010
Yahara H, Satoh T, Miyagishi C, Yokozeki H	Increased expression of CRTH2 on eosinophils in allergic skin diseases.	J Eur Acad Dermmatol Venereol.	24	75-6	2010
Yoshiki R, Kabashima K, Sakabe J, Sugita K, Bito T, Nakamura M, Malissen B, Tokura Y.	The mandatory role of IL-10-producing and OX40 ligand-expressing mature Langerhans cells in local UVB-induced immunosuppression.	J Immunol.	184	5670-7	2010
Zhang Q, Andoh T, Konno M, Lee J.B, Hattori M, Kuraishi Y.	Inhibitory effect of methanol extract of Ganoderma lucidum on acute itch-associated responses in mice. Biol.	Pharm. Bull.	33	909-11	2010
安東嗣修	皮膚における痒み因子	アレルギー・免疫	17	1506-10	2010
烏山 一	吸血ダニに対する抵抗性、好塩基球が鍵を握っていた	Medical bio	7 (6)	52-5	2010
烏山 一	急性ならびに慢性アレルギー発症における好塩基球の新たな役割	炎症と免疫	18(1)	23-27	2010
烏山 一	好塩基球が主役を演じる IgE 依存性慢性アレルギー炎症	IgE practice in asthma		1-5	2010
室田浩之, 片山一朗, 谷内一彦	かゆみを伴う皮膚疾患における抗ヒスタミン薬の選択 薬剤のパフォーマンスに及ぼす影響を考慮して	診療と新薬	47 巻 9 号	964-73	2010
室田浩之, 片山一朗	【痒みのメカニズムアップデート】痒みと仕事の生産性	アレルギー・免疫	17 巻 9 号	1539-44	2010
Andoh T, Haza S, Saito A, Kuraishi Y.	Involvement of leukotriene B4 in spontaneous itch-related behavior in NC mice with atopic dermatitis-like skin lesions.	Exp. Dermatol.	20(11)	894-8	2011
Andoh T, Kuwazono T, Lee JB, Kuraishi Y.	Gastrin-releasing peptide induces itch-related responses through mast cell degranulation in mice.	Peptides.	32(10)	2098-103	2011
Arase N, Wataya-Kaneda M, Oiso N, Tanemura A, Kawada A, Suzuki T, Katayama I.	Repigmentation of leukoderma in a piebald patient associated with a novel c-KIT gene mutation, G592E, of the tyrosine kinase domain.	J Dermatol Sci.	64(2)	147-9	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Azukizawa H, Döhler A, Kanazawa N, Nayak A, Lipp M, Malissen B, Autenrieth I, Katayama I, Riemann M, Weih F, Berberich-Siebelt F, Lutz MB	Steady state migratory RelB+ langerin+ dermal dendritic cells mediate peripheral induction of antigen-specific CD4+ CD25+ Foxp3+ regulatory T cells.	Eur J Immunol.	41(5)	1420-34	2011
Fukamachi S, Bito T, Shiraishi N, Kobayashi M, Kabashima K, Nakamura M, Tokura Y.	Modulation of semaphorin 3A expression by calcium concentration and histamine in human keratinocytes and fibroblasts.	J Dermatol Sci.	61(2)	118-23	2011
Fukamachi S, Mori T, Sakabe J-I, Shiraishi N, Kuroda E, Kobayashi M, Bito T, Kabashima K, Nakamura M, Tokura Y	Topical Cholecystokinin depresses itch-associated scratching behavior in mice.	J Invest Dermatol.	131	956-61	2011
Gotoh Y, Andoh T, Kuraishi Y	Noradrenergic regulation of itch transmission in the spinal cord mediated by α-adrenoceptors.	Neuropharmacol	61(4)	825-31	2011
Gotoh Y, Omori Y, Andoh T, Kuraishi Y.	Tonic inhibition of allergic itch signaling by the descending noradrenergic system in mice.	J. Pharmacol. Sci.	115(3)	417-20	2011
Gotoh Y, Andoh T, Kuraishi Y.	Clonidine inhibits itch-related response through stimulation of α 2-adrenoceptors in the spinal cord in mice.	Eur. J. Pharmacol.	650	215-9	2011
Hanafusa T, Azukizawa H, Kitaba S, Murota H, Umegaki N, Terao M, Sano S, Nakagiri T, Okumura M, Katayama I.	Diminished regulatory T cells in cutaneous lesions of thymoma-associated multi-organ autoimmunity: a newly described paraneoplastic autoimmune disorder with fatal clinical course.	Clin Exp Immunol.	166(2)	164-70	2011
Hanafusa T, Igawa K, Azukizawa H, Katayama I.	Acute generalized exanthematous pustulosis induced by topical diphenhydramine.	Eur J Dermatol.	21(6)	994-5	2011
Hanafusa T, Igawa K, Takagawa S, Yahara H, Harada J, Tani M, Sawada Y, Katayama I.	Erythroderma as a paraneoplastic cutaneous disorder in systemic anaplastic large cell lymphoma.	J Eur Acad Dermatol Venereol.	[in press]		2011
Hanafusa T, Tamai K, Umegaki N, Yamaguchi Y, Fukuda S, Nishikawa Y, Yaegashi N, Okuyama R, McGrath JA, Katayama I.	The course of pregnancy and childbirth in three mothers with recessive dystrophic epidermolysis bullosa.	Clin Exp Dermatol.	37(1)	10-4	2011
Hayashi H, Kohno T, Yasui K, Murota H, Kimura T, Duncan GS, Nakashima T, Yamamoto K, Katayama I, Ma Y, Chua KJ, Suematsu T, Shimokawa I, Akira S, Kubo Y, Mak TW, Matsuyama T.	Characterization of dsRNA-induced pancreatitis model reveals the regulatory role of IFN regulatory factor 2 (Irf2) in trypsinogen5 gene transcription.	Proc Natl Acad Sci U S A.	108(46)	18766-71	2011
Hino R, Kabashima R, Kawakami C, Sugita K, Nakamura M, Tokura Y.	Circulating Th17 cell fluctuation in psoriatic patients treated with topical calcipotriol and betamethasone butyrate propionate.	J EurAcadDermat olVnereol.	25	242-4	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hirakawa S, Tanemura A, Mori H, Katayama I, Hashimoto K.	Multiple lymphadenopathy as an initial sign of extramammary Paget disease.	Br J Dermatol.	164(1)	200-3	2011
Honda T, Otsuka A, Tanizaki H, Minegaki Y, Nagao K, Waldmann H, Tomura M, Hori S, Miyachi Y, Kabashima K.	Enhanced murine contact hypersensitivity by depletion of endogenous regulatory T cells in the sensitization phase.	J Dermatol Sci	61	144-7	2011
Hosoya K, Satoh T, Yamamoto Y, Saeki K, Igawa K, Okano M, Moriya T, Imamura O, Nemoto Y, Yokozeki H.	Gene silencing of STAT6 with siRNA ameliorates contact hypersensitivity and allergic rhinitis.	Allergy .	66	124-31	2011
Igawa K, Satoh T, Yokozeki H.	Possible association of Henoch-Schoenlein purpura in adults with odontogenic focal infection.	Int J Dermatol.	50	277-9	2011
Imai A, Takayama K, Satoh T, Katoh T, Yokozeki H.	Ingrown nail and pachyonychia of the great toes impaire lower limb function: improvement of limb dysfunction by medical foot care treatment.	Int J Dermatol.	50	215-20	2011
Ishikawa Y, Kobayashi K, Yamamoto M, Nakata K, Takagawa T, Funada Y, Kotani Y, Karasuyama H, Yoshida M, and Nishimuar Y.	Antigen-specific IgG ameliorates allergic airway inflammation via Fcγ receptor IIB on dendritic cells.	Respir. Res.	12	42	2011
Ito Y, Satoh T, Takayama K, Miyagishi C, Walls AF, Yokozeki H.	Basophil recruitment and activation in inflammatory skin diseases.	Allergy	66	1107-13	2011
Itoi S, Tanemura A, Nishioka M, Sakimoto K, Iimuro E, Katayama I.	Evaluation of the clinical safety and efficacy of a newly developed 308-nm excimer lamp for vitiligo vulgaris.	J Dermatol.	[in press]		2011
Jönsson F, Mancardi D.A, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, Shimizu T, Daëron M and Bruhns P.	Mouse and human neutrophils induce anaphylaxis. J. Clin.	Invest.	121	1484-96	2011
Kabashima R, Sugita K, Sawada Y, Hino R, Nakamura M, Tokura Y.	Increased circulating Th17 frequencies and serum IL-22 levels in patients with acute generalized exanthematouspustulosis.	J EurAcadDermatolVenereol	25	485-8	2011
Karasuyama H, Mukai K, Obata K, Tsujimura Y, and Wada T.	Nonredundant roles of basophils in immunity.	Annu. Rev. Immunol.	29	45-69	2011
Karasuyama H, Wada T, Yoshikawa S, and Obata K.	Emerging roles of basophils in protective immunity against parasites.	Trends Immunol.	32	125-30	2011
Karasuyama, H, Obata, K, Wada T, Tsujimura Y, and Mukai K.	Newly appreciated roles for basophils in allergy and protective immunity.	Allergy	66	1133-41	2011
Katayama I, Kohno Y, Akiyama K, Ikezawa Z, Kondo N, Tamaki K, Kouro O.	Japanese guideline for atopic dermatitis.	Japanese Society of Allergology.	60(2)	205-20	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kato K, Satoh T, Nishizawa A, Yokozeki H.	Psoriasiform drug eruption due to abatacept.	Acta Derm Venereol.	91	362-3	2011
Kitaba S, Matsui S, Iimuro E, Nishioka M, Kijima A, Umegaki N, Murota H, Katayama I.	Four Cases of Atopic Dermatitis Complicated by Sjögren's Syndrome: Link between Dry Skin and Autoimmune Anhidrosis.	Allergol Int.	60(3)	387-91	2011
Kiyohara E, Tamai K, Katayama I, Kaneda Y	The combination of chemotherapy with HVJ-E containing Rad51 siRNA elicited diverse anti-tumor effects and synergistically suppressed melanoma.	Gene Ther.	[in press]		2011
Matsushima Y, Satoh T, Yamamoto Y, Nakamura M, Yokozeki H.	Distinct roles of prostaglandin D2 receptors in chronic skin inflammation.	Mol Immunol.	49(1-2)	304-10	2011
Moniaga CS, Egawa G, Doi H, Miyachi Y, Kabashima K.	Histamine modulates the responsiveness of keratinocytes to IL-17 and TNF-alpha through the H1-receptor.	J Dermatol Sci.	61	79-81	2011
Murakami Y, Matsui S, Kijima A, Kitaba S, Murota H, Katayama I.	Cedar pollen aggravates atopic dermatitis in childhood monozygotic twin patients with allergic rhino conjunctivitis.	Allergol Int.	60(3)	397-400	2011
Murakami Y, Wataya-Kaneda M, Terao M, Az.ukizawa H, Murota H, Nakata Y, Katayama I.	Peculiar distribution of tumorous xanthomas in an adult case of erdheim-chester disease complicated by atopic dermatitis.	Case Rep Dermatol.	3(2)	107-12	2011
Murota H, Katayama I.	Assessment of antihistamines in the treatment of skin allergies.	Curr Opin Allergy Clin Immunol.	11(5)	428-37	2011
Murota H, Katayama I.	Lichen aureus responding to topical tacrolimus treatment.	J Dermatol.	38(8)	823-5	2011
Nakagawa Y, Takamatsu H, Okuno T, Kang S, Nojima S, Kimura T, Kataoka TR, Ikawa M, Toyofuku T, Katayama I, Kumanogoh A.	Identification of semaphorin 4B as a negative regulator of basophil-mediated immune responses.	J Immunol.	186(5)	2881-8	2011
Nakamizo S, Kurosawa M, Sawada Y, Tokura Y, Miyachi Y, Kabashima K.	A case of cholinergic urticaria associated with acquired generalized hypohidrosis and reduced acetylcholine receptors: cause and effect?	Clin Exp Dermatol.	36	559-60	2011
Namiki T, Tanemura A, Valencia JC, Coelho SG, Passeron T, Kawaguchi M, Vieira WD, Ishikawa M, Nishijima W, Izumo T, Kaneko Y, Katayama I, Yamaguchi Y, Yin L, Polley EC, Liu H, Kawakami Y, Eishi Y, Takahashi E, Yokozeki H, Hearing VJ.	AMP kinase-related kinase NUAK2 affects tumor growth, migration, and clinical outcome of human melanoma.	Proc Natl Acad Sci U S A.	108(16)	6597-602	2011
Nishikado H., Mukai, K, Kawano Y, Minegishi Y, and Karasuyama H.	NK cell-depleting anti-asialo GM1 Ab exhibits a lethal off-target effect on basophils in vivo.	J. Immunol.	186	5766-5771	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nishioka M, Tanemura A, Yamanaka T, Umegaki N, Tani M, Katayama I, Takemasa I, Sekimoto M, Tomita K, Tamai N.	A case of giant squamous cell carcinoma of the buttock possibly arose from syringocystadenoma and invaded to the rectum.	J Skin Cancer.	2011	213406	2011
Nishioka M, Tani M, Murota H, Katayama I.	Eosinophilic pyoderma gangrenosum with pulmonary and oral lesions preceded by eosinophilic pneumonia: Unrecognized syndromic manifestations?	Eur J Dermatol.	21(4)	631-2	2011
Otsuka A, Honda T, Doi H, Miyachi Y, Kabashima K	An H1-histamine receptor antagonist decreases serum interleukin-31 levels in patients with atopic dermatitis.	Br J Dermatol.	164	455-6	2011
Otsuka A, Kubo M, Honda T, Egawa G, Nakajima S, Tanizaki H, Kim B, Matsuoka S, Watanabe T, Nakae S, Miyachi Y, Kabashima K.	Requirement of interaction between mast cells and skin dendritic cells to establish contact hypersensitivity.	PLoS One.	6(9)	e25538	2011
Otsuka A, Miyachi Y, Kabashima K.	Narrowband ultraviolet B phototherapy decreased CCR4+ CD8+ T cells in a patient with palmoplantar pustulosis.	J Eur Acad Dermatol Venereol.	25	495-6	2011
Otsuka A, Tanioka M, Nakagawa Y, Honda T, Ikoma A, Miyachi Y, Kabashima K.	Effects of cyclosporine on pruritus and serum IL-31 levels in patients with atopic dermatitis.	Eur J Dermatol.	21	816-7	2011
Park K, Mori M, Nakamura M, Tokura Y.	Increased expression of mRNAs for IL-4, IL-17, IL-22 and IL-31 in skin lesions of subacute and chronic forms of prurigo.	Eur J Dermatol.	21	135-6	2011
Porcherie A, Mathieu,C, Peronet R, Schneider E, Claver J, Commere P-H, Kiefer-Biasizzo H, Karasuyama H, Milon G, Dy M, Kinet J-P, Louis J, Blank U, and Mecheri S.	Critical role of the neutrophil-associated high-affinity receptor for IgE in the pathogenesis of experimental cereberal malaria.	J. Exp. Med.	208	2225-6	2011
Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, Agematsu K, Yamada M, Kawamura N, Ariga T, Tsuge I, Nonoyama S, Karasuyama H, and Minegishi Y.	Defective IL-10 signaling in hyper-IgE syndrome results in impaired generation of tolerogenic dendritic cells and induced regulatory T cells.	J. Exp. Med.	208	235-49	2011
Satoh T, Ito Y, Miyagishi C, Yokozeki H.	Basophils infiltrate skin lesions of eosinophilic pustular folliculitis (Ofuji's disease).	Acta Derm Venereol	91(3)	371-2	2011
Tamai K, Yamazaki T, Chino T, Ishii M, Otsuru S, Kikuchi Y, Iinuma S, Saga K, Nimura K, Shimbo T, Umegaki N, Katayama I, Miyazaki J, Takeda J, McGrath JA, Uitto J, KanedaY.	PDGFRalpha-positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia.	Proc Natl Acad Sci U S A.	108(16)	6609-14	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tanaka T, Satoh T, Tanaka A, Yokozeki H.	Congenotal insensivitity to pain with anhidrosis: a case with preserved itch sensation to histamine and partial pain sensation.	Br J Dermatol	[in press]		2011
Terao M, Ishikawa A, Nakahara S, Kimura A, Kato A, Moriwaki K, Kamada Y, Murota H, Taniguchi N, Katayama I, Miyoshi E.	Enhanced Epithelial-Mesenchymal Transition-like Phenotype in N-Acetylglucosaminyltransferase V Transgenic Mouse Skin Promotes Wound Healing.	J Biol Chem.	286(32)	28303-11	2011
Terao M, Matsui S, Katayama I.	Two cases of refractory discoid lupus erythematosus successfully treated with topical tocoretinate.	Dermatol Online J.	17(4)	15	2011
Terao M, Murota H, Kimura A, Kato A, Ishikawa A, Igawa K, Miyoshi E, Katayama I.	11β-hydroxysteroid dehydrogenase-1 is a novel regulator of skin homeostasis and a candidate target for promoting tissue repair.	PLoS One.	6(9)	e25039	2011
Terao M, Nishida K, Murota H, Katayama I.	Clinical effect of tocoretinate on lichen and macular amyloidosis.	J Dermatol.	38(2)	179-84	2011
Ugajin T, Satoh T, Kanamori T, Aritake K, Urade Y, Yokozeki H.	FcεRI, but not FcγR, signals induce prostaglandin D2 and E2 production from basophils.	Am J Pathol.	179	775-82	2011
Umegaki N, Nakano H, Tamai K, Mitsuhashi Y, Akasaka E, Sawamura D, Katayama I.	Vörner type palmoplantar keratoderma: novel KRT9 mutation associated with knuckle pad-like lesions and recurrent mutation causing digital mutilation.	Br J Dermatol.	165(1)	199-201	2011
Wataya-Kaneda M, Tanaka M, Nakamura A, Matsumoto S, Katayama I.	A topical combination of rapamycin and tacrolimus for the treatment of angiofibroma due to tuberous sclerosis complex (TSC): a pilot study of nine Japanese patients with TSC of different disease severity.	Br J Dermatol.	165(4)	912-6	2011
Yamagishi H, Mochizuki Y, Hamakubo T, Obata K, Ugajin T, Sato S, Kawano Y, Minegishi Y, and Karasuyama H.	Basophil-derived mouse mast cell protease 11 induces microvascular leakage and tissue edema in a mast cell-independent manner. Biochem. Biophys.	Res. Commun.	415	709-13	2011
Yamamoto T, Katayama I.	Vascular changes in bleomycin-induced scleroderma.	Int J Rheumatol.	2011	270938	2011
Yamamoto Y, Otani S, Hirai H, Nagata K, Aritake K, Urade Y, Narumiya S, Yokozeki H, Nakamura M, Satoh T.	Dual functions of prostaglandin D2 in murine contact hypersensitivity via DP and CRTH2.	Am J Pathol.	179	302-14	2011
烏山 一	アレルギーと生体防御における好塩基球の新たな役割	アレルギー・免疫	18(4)	42-9	2011
烏山 一	最近クローズアップされた好塩基球の重要性	感染・炎症・免疫	40(4)	12-21	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
室田浩之, 北場俊, 片山一朗他	大阪大学関連施設を中心としたアトピー性皮膚炎患者の生活習慣実態調査研究	J Environ Dermatol Cutan Allergol.	5	103-14	2011
小畑一茂, 石川亮, 烏山 一	「慢性アレルギーと好塩基球」慢性炎症—多様な疾患の基盤病態	実験医学増刊号	29(10)	151-5	2011
田村忠史, 室田浩之, 片山一朗	オロパタジンによる痒みと表皮内神経線維の伸長の制御	アレルギーと神経ペプチド	7	32-6	2011
片山一朗	アトピー性皮膚炎の診断と治療	日本医師会雑誌	140(5)	945-58	2011
片山一朗	アトピー性皮膚炎の診療ガイドライン	アレルギー・免疫	18(10)	10-20	2011
片山一朗	アトピー性皮膚炎の病因	日本医師会雑誌	140(5)	978-82	2011
片山一朗	包括的カユミ対策をスキンケアはアレルギーマーチを阻止できるか？	日本小児皮膚科学会雑誌	30(1)	1-7	2011
片山一朗, 古江増隆, 川島眞他	アトピー性皮膚炎患者における前向きアンケート調査（第2報）	臨床皮膚科	65(1)	83-92	2011
北場俊, 室田浩之, 熊ノ郷卓之他	【アレルギー疾患と睡眠障害】臨床医学からのアプローチ 蕁麻疹・アトピー性皮膚炎と睡眠障害	アレルギー・免疫	18	230-5	2011
Kanai Y, Satoh T, Yokozeki H.	Impaired expression of Tim-3 on Th17 and Th1 cells in psoriasis.	Acta Derm-Venereol	[in press]		2012
Kitaba S, Murota H, Terao M, Azukizawa H, Terabe F, Shima Y, Fujimoto M,Tanaka T, Naka T, Kishimoto T, Katayama I.	Blockade of interleukin-6 receptor alleviates disease in mouse model of scleroderma.	Am J Pathol.	180	165-76	2012
Nakahigashi K, Doi H, Otsuka A, Hirabayashi T, Murakami M, Urade Y, Tanizaki H, Egawa G, Miyachi Y, Kabashima K.	PGD2 induces eotaxin-3 via PPARγ from sebocytes: A possible pathogenesis of eosinophilic pustular folliculitis.	J Allergy Clin Immunol.	129(2)	536-43	2012
Nakamizo S, Egawa G, Miyachi Y, Kabashima K.	Cholinergic urticaria: pathogenesis-based categorization and its treatment options.	J Eur Acad Dermatol Venereol.	26	114-6	2012
Ogata A, Umegaki N, Katayama I, Kumanogoh A, Tanaka T.	Psoriatic arthritis in two patients with an inadequate response to treatment with tocilizumab.	Joint Bone Spine.	79(1)	85-7	2012
Otsuka A, Miyagawa-Hayashino A, Walls A, Miyachi Y, Kabashima K.	Comparison of basophil infiltration into the skin between eosinophilic pustular folliculitis and neutrophilic folliculitis.	J Eur Acad Dermatol Venereol.	26(4)	527-9	2012
Sawaguchi M, Tanaka S, Nakatani Y, Harada Y, Mukai K, Matsunaga Y, Ishiwata K, Oboki K, Kambayashi T, Watanabe N, Karasuyama H, Nakae S, Inoue H, and Kubo M.	Role of mast cells and basophils in IgE responses and in allergic airway hyperresponsiveness.	J. Immunol.	188		2012
Sekine R, Satoh T, Takaoka A, Saeki K, Yokozeki H.	Anti-pruritic effects of topical crotamiton, capsaicin, and a corticosteroid on pruritogen-induced scratching behavior.	Exp Dermatol.	21(3)	201-4	2012
Yu R, Satoh T, Wakabayashi T, Ueda N, Yokozeki H.	Disseminated BCG infection in severe combined immunodeficiency.	Acta Derm Venereol.	92(2)	158-9	2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Igawa K, Satoh T, Yokozeki H.	Anaphylactoid purpura in adults associated with odontogenic infection.	Int J Dermatol	[in press]		
Inazawa M, Satoh T, Yokozeki H.	Hyperkeratotic variant of inflammatory disseminated superficial porokeratosis with lichenoid reaction and extensive amyloid deposition.	Int J Dermatol.	[in press]		

Ⅳ．研究成果の刊行物・別刷

Overproduction of IgE Induces Macrophage-Derived Chemokine (CCL22) Secretion from Basophils

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Macrophage-derived chemokine (MDC) CCL22 is a potent chemoattractant for Th2 cells and has been implicated in Th2-predominant allergic inflammation. In the present study, we demonstrated that basophils produce MDC in response to monomeric IgE. In trinitrophenyl (TNP)-IgE transgenic mice, serum levels of MDC were persistently higher than in wild-type mice. The i.v. administration of TNP-specific IgE to wild-type mice transiently induced an elevation in serum MDC, which appeared to be mediated by FcεRI, as no increase in serum MDC was observed after IgE administration in FcRγ (−/−) mice. However, the IgE-mediated increase in MDC was observed in mast cell-deficient mice. Freshly isolated bone marrow cells and bone marrow-derived basophils secreted MDC in response to TNP-IgE without Ag stimulation. Furthermore, MDC production was not observed in bone marrow-derived basophils isolated from FcRγ (−/−) mice. IgE activated Lyn and ERK 1/2 in bone marrow-derived basophils. Treatment of TNP-IgE transgenic mice with a basophil-depletion Ab (Ba103) resulted in decreased serum MDC levels. Thus, IgE appears to be capable of stimulating basophils to produce MDC in the absence of a specific Ag, which may contribute to IgE-mediated and/or Th2-predominant allergic inflammation. *The Journal of Immunology*, 2008, 181: 5653–5659.

I n recent years, a considerable amount of attention has been paid to the roles of chemokines that attract effector cells in inflammatory responses. Macrophage-derived chemokine (MDC)[3] CCL22 is a C-C chemokine secreted by a variety of cells, such as macrophages, monocytes, dendritic cells, and B cells (1, 2). MDC acts as a chemoattractant for CCR4-expressing cells (3, 4), including Th2-type lymphocytes, and it has been implicated in allergic diseases. MDC production is increased in asthmatic patients (5, 6), and the blocking of MDC causes an impaired reaction in asthmatic models in mice (7). Serum levels of MDC are increased and correlated with skin disease activity in patients with atopic dermatitis (8, 9). However, the mechanisms leading to the persistent production of MDC in allergic diseases are not fully understood.

Chronic allergic skin diseases, such as atopic dermatitis, are frequently accompanied by increased levels of serum IgE. IgE is thought to evoke skin inflammation via the activation of mast cells. Mast cells degranulate a variety of chemical mediators, including histamines and prostaglandins, by the interaction of IgE and Ag, resulting in the immediate-type reactions (ITR), such as the wheal-and-flare skin response. The late-phase re-

sponses (LPR) occur hours after the ITR. The LPR is histologically characterized by a dermal cellular infiltrate comprised of lymphocytes, neutrophils, and eosinophils. The LPR is also a mast cell-dependent reaction that is probably initiated by mast cell-derived cytokines, such as TNF-α and IL-4 (10), and possibly by several chemokines; the cytokines stimulate the expression of cell adhesion molecules on dermal endothelial cells, and the chemokines mediate the chemoattraction of effector cells. A recent study demonstrated that transgenic mice overexpressing IgE exhibited a strong "third-phase reaction" following the ITR and LPR (11, 12). This third-phase reaction lasts for several days and is histologically characterized by a dense cellular infiltrate comprised of lymphocytes and eosinophils with epidermal hyperplasia and hyperkeratosis (11), which indicate a chronic allergic inflammation. In addition, the striking finding was that basophils, but not mast cells, were essential for the development of the IgE-mediated third-phase reaction (12). These findings unveiled novel and overlooked pathological roles of IgE and basophils in chronic allergic inflammation.

Both basophils and mast cells express the αβγ2 form of FcεRI; the α-chain is responsible for binding the Fc portion of IgE, whereas the β- and γ-chains contain the tyrosine-based activation motifs responsible for transducing the activation signal in the cells (13, 14). The traditional understanding of cell activation via FcεRI has been that IgE binds to FcεRI, resulting in cell priming and the increased surface density of FcεRI; but IgE binding does not evoke the activation events, such as the release of preformed granule-associated mediators, newly synthesized arachidonic acid metabolites, and cytokines. The full activation of FcεRI requires cross-linking of the α-chain, which is mediated by binding to multivalent allergen through surface IgE. Nevertheless, recent studies with mast cells revealed that monomeric IgE in the absence of a specific Ag can activate several signaling events, leading to prolonged cell survival, degranulation, and the production of cytokines such as IL-6 and

TNF-α (15–22). These observations have changed the conceptual understanding of IgE binding to cells from a pre-activating event to a full-activating event. Thus, it is conceivable that high levels of IgE may persistently stimulate mast cells and/or basophils to release inflammatory mediators before exposure to allergens and may modify or reinforce the subsequent allergic reactions evoked by allergen challenge. However, not all monoclonal IgE molecules induce complete cell activation. IgEs display heterogeneity in that different IgE molecules induce varied levels of activation. Highly cytokinergic IgEs induce cytokine secretion and other activation events including degranulation, whereas other IgEs do not efficiently lead to these events (16, 17, 19, 20).

In the present study, we found that mice overexpressing trinitrophenyl (TNP)-specific IgE have unique properties characterized by a higher production of MDC than in wild-type mice. We attempted to elucidate the mechanisms of MDC generation and demonstrated that basophils seem to be persistently generating MDC by the binding of TNP-IgE to FcεRI on their surface in the absence of an Ag.

Materials and Methods

Mice

BALB/c, C57BL/6, C3H/HeJ, and WBB6F$_1$-Sl/Sld mice were purchased from Sankyo Labo Service. FcRγ-chain$-/-$ C57BL/6 mice (23) were purchased from The Jackson Laboratory. TNP-specific IgE transgenic mice were described previously (24). Mice were maintained under specific pathogen-free conditions in our animal facility. The use of animals was in full compliance with the Committee for Animal Experiments of Tokyo Medical and Dental University.

Preparation of monomeric IgE

TNP-specific IgE was derived from the IGEL b4 B cell hybridoma (American Type Culture Collection) (25) by collecting in vitro culture supernatants. In some experiments, IgE was obtained from ascites of CD1 (ICR)-nu/nu mice by i.p. injection of the hybridoma. Supernatants were subjected to precipitation with 50% saturated ammonium sulfate and dialysis in PBS. Dinitrophenyl (DNP)-specific IgE (SPE-7) was obtained from Sigma-Aldrich. Monomeric IgE was prepared by removing aggregates with ultracentrifugation at 100,000 $\times$ g for 1 h at 4°C (18).

Cell preparation

Bone marrow-derived basophils (BMBa) were obtained according to the protocol reported previously (26). In brief, bone marrow cells were aspirated from 6- to 8-wk-old mice, and Lin ($-$) bone marrow progenitor cells were isolated with Spin Sep Ab mixture (StemCell Technologies). These cells were cultured with rIL-3 (Wako Pure Chemical Industries, Ltd.; 10 ng/ml) for 10 days. The c-kit ($-$) basophil-like cells were obtained by negative selection with biotin-conjugated anti-mouse c-kit Ab (eBioscience) and streptavidin microbeads (Milteni Biotec) by using magnetic cell sorting. FcεRI ($+$)/c-kit ($-$) cells constituted ~70–80% of these cell suspensions as assessed by flow cytometry (data not shown).

Bone marrow-derived mast cells (BMMC) were prepared by culturing bone marrow cells in RPMI 1640 medium that contained 10% FCS, 10 mM nonessential amino acids, 10 mM sodium pyruvate, 25 mM HEPES buffer, 50 μM 2-ME, and rIL-3 (10 ng/ml) for 5 wk. More than 98% of BMMC were positive for FcεRI and c-kit, as assessed by flow cytometric analysis with FITC-labeled anti-mouse FcεRI Ab (MAR-1; eBioscience) and PE-labeled anti-c-kit Ab (BD Biosciences).

Peritoneal mast cells were prepared as described previously (27, 28) with some modifications. Briefly, mice were injected i.p. with 5 ml of RPMI 1640. The peritoneal lavages were collected after gentle massages of abdomen. Cells were cultured for 10–14 days in the presence of 10 ng/ml rIL-3 and 30 ng/ml stem cell factor (PeproTech). The purity of mast cells was more than 95%, as determined by the flow cytometric analysis for c-kit and FcεRI.

Stimulation of cells with monomeric IgE

BMBa or BMMC (5 $\times$ 10^5 cells/1.5 ml) were incubated for 24 h with the indicated concentrations of IgE in RPMI 1640 that contained 10% FCS. Supernatants were collected and stored at -20°C until use.

Preparation of TNP-OVA

OVA (Sigma-Aldrich; 50 mg/1.5 ml) was mixed with 2.5 ml of 0.1 M Na$_2$BO$_3$ buffer (pH 9.3) and incubated with 1 ml of 5% 2,4,6-trinitrobenznesulfonic acid (Nacalai Tesque) and 10 μl of 1 M Na$_2$CO$_3$ overnight at room temperature. OVA conjugated with more than 11 molecules of TNP per protein was used for the experiments after dialysis with PBS (11, 12).

Stimulation of cells with Ags

Cells were primed overnight with 0.5 μg/ml TNP-IgE or 0.5 μg/ml DNP-IgE, then washed and stimulated with 20 ng/ml TNP-OVA or DNP-HSA (Sigma-Aldrich) for 24 h. Supernatants were collected and stored at -20°C until use.

Degranulation assay

Degranulation was assessed by using a previously reported method to measure the release of β-hexosaminidase (29, 30). Cells were washed with Tyrode's salt solution (pH 7.4) containing 0.04% BSA, 10 mM HEPES, and 10 ng/ml IL-3. Cells were seeded at 5 $\times$ 10^5 cells/1.5 ml on a 24-well plate and incubated for 20 min at 37°C. They were stimulated with TNP-IgE or DNP-IgE for 30 min. At the end of the incubation period, the plate was transferred to ice, and the cells were centrifuged at 500 $\times$ g for 10 min at 4°C. After collection of the supernatant, the pellets were solubilized in the original volume of HEPES-Tyrode's buffer containing 0.5% Triton-X-100. Next, 50 μl of sample (in duplicate) was incubated with 50 μl of 1 mM p-nitrophenyl-N-acetyl-β-D-glucosamide (Sigma-Aldrich) dissolved in 0.1 M citrate buffer (pH 5.0) in a 96-well microtiter plate at 37°C for 1.5 h. The reaction was stopped with 200 μl/well of 0.1 M carbonate buffer (pH 10.5). The plate was read at 405 nm in an ELISA reader. The net percentage of β-hexosaminidase release was calculated as follows: (β-hexosaminidase in supernatants)/(β-hexosaminidase in supernatant + β-hexosaminidase in pellet) $\times$ 100 (%).

Real-Time PCR

Total cellular RNA was isolated by using Isogen (Nippon Gene). Twenty microliters of reverse transcription mix consisted of 8 μl of 5$\times$ buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 50 mM DTT, 15 mM MgCl$_2$), 4 μl of hexanucleotide mixture (62.5 A_{260} U/ml, Roche Diagnostics), 2 μl of dNTPs (2.5 mM each), 4 μl of 20 U/μl human placenta RNase inhibitor (Takara Bio), and 2 μl of 200 U/μl reverse transcriptase (Moloney murine leukemia virus; Takara Bio). The reverse transcription mixture was dispensed at 20 μl/tube with 20 μl of 40 ng/μl total RNA, vortex-mixed, and then incubated at 37°C for 60 min. Reverse transcriptase was inactivated at 70°C for 10 min, and then the samples were stored at -80°C.

Quantitative RT-PCR was performed by monitoring in real time the increase in fluorescence of the SYBR Green dye (Brilliant SYBR Green QPCR Master Mix; Stratagene) with the Mx3000P Real-Time PCR system (Stratagene). The primers for PCR were 5'-AAATGGTGAAGGTCGGT GTG-3' and 5'-TGAAGGGGTCGTTGATGG-3' for mouse GAPDH, and 5'-TCATGGCTACCCTGCGTGTC-3' and 5'-CCTTCACTAAACGTG ATGGCAGAG-3' for mouse MDC.

Measurement of MDC/CCL22

Serum was collected by centrifugation of blood obtained from the retroorbital plexus. The concentration of MDC was measured with ELISA kits (R&D Systems).

Immunoblotting analysis

Cells stimulated with IgE and/or Ags were washed with ice-cold PBS ($-$) and lysed in 1% Triton-containing lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na$_3$VO$_4$, 1 μg/ml leupeptin, and 1 mM PMSF). Cell lysates were analyzed by SDS-PAGE followed by immunoblotting. Proteins reactive with primary Abs, such as anti-phospho Src family Ab (Tyr^{416}) (Cell Signaling Technology), anti-Lyn Ab (Santa Cruz Biotechnology), anti-phospho-p42/44 MAPK Ab (Thr^{202}/Tyr^{204}; Cell Signaling Technology), and anti-p43/44 MAPK Ab (Cell Signaling Technology),
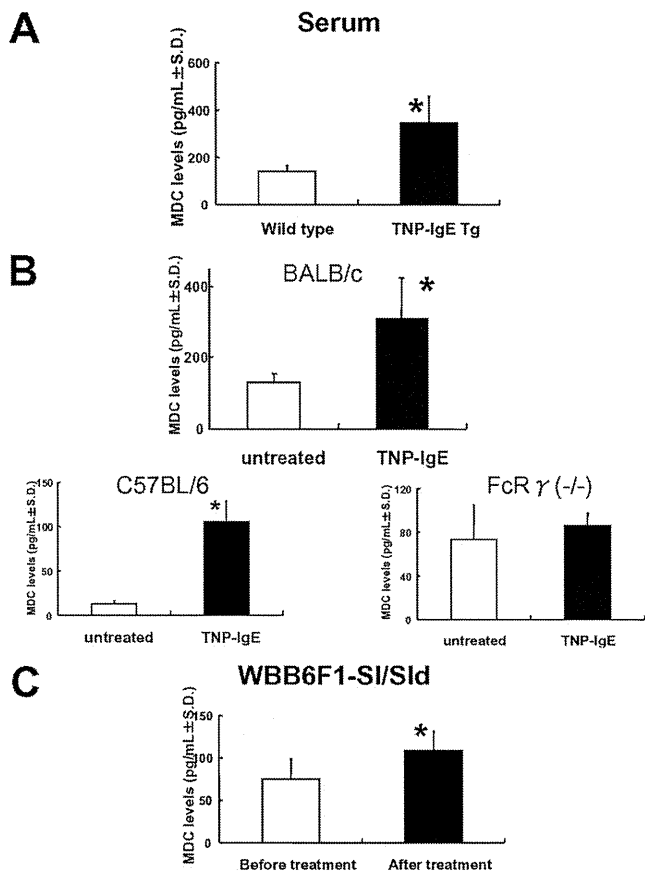
FIGURE 1. Serum MDC is increased by TNP-IgE. A, In TNP-IgE transgenic mice, levels of MDC are persistently higher than in wild-type BALB/c mice. B, Naive mice were injected i.v. with TNP-IgE, and the serum MDC levels were measured 24 h later. BALB/c mice produced MDC in response to IgE injection. FcRγ (−/−) mice did not respond to IgE, whereas an increase in MDC was observed in wild-type C57BL/6 mice. C, Mast cell-deficient WBB6F₁-Sl/Sld mice also produced MDC in response to IgE injection. Each group consisted of at least five mice. ∗, p < 0.05.
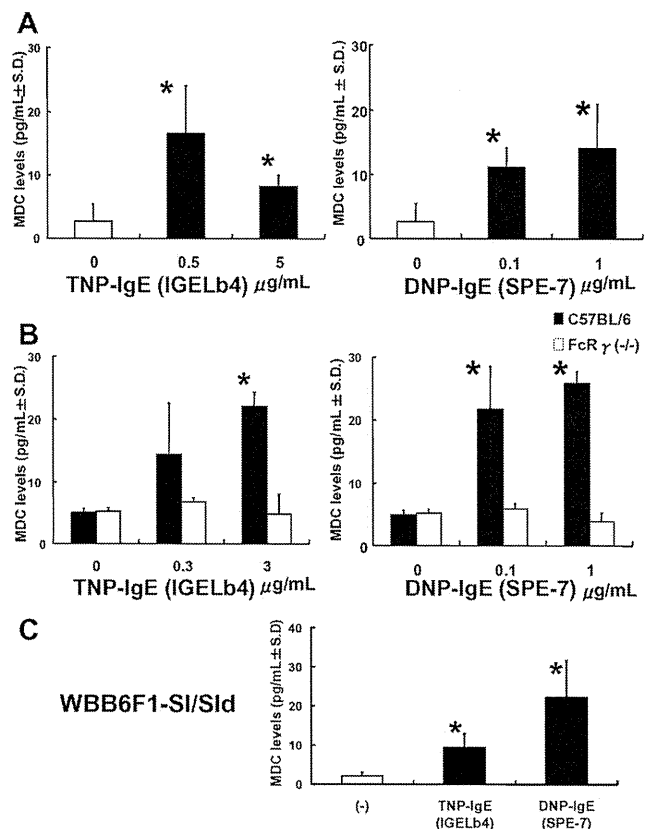

FIGURE 2. MDC production from freshly isolated bone marrow cells in vitro. A, Bone marrow cells freshly prepared from BALB/c mice were stimulated with TNP-IgE or DNP-IgE for 24 h. Both types of IgE induced MDC production. B, Unlike bone marrow cells from wild-type C57BL/6 mice, bone marrow cells from FcRγ (−/−) mice failed to produce MDC in response to IgE. C, MDC secretion was observed in bone marrow cells from mast cell-deficient mice. Representative results of at least three separate experiments are shown. ∗, p < 0.05.

were visualized with an HRP-conjugated secondary Ab and ECL reagents (Amersham Biosciences).

Statistical analyses

The Student's *t* test was used to assess the statistical significance of differences between mean values. Values of p < 0.05 were considered statistically significant.

Results

TNP-IgE induces serum MDC production in vivo

Fig. 1A shows MDC levels in TNP-specific IgE transgenic mice. Serum levels of MDC in TNP-IgE transgenic mice were persistently higher than those in wild-type BALB/c mice. To examine the mechanisms of increased MDC production in TNP-IgE transgenic mice, we i.v. administered TNP-IgE (300 μg/mouse) into wild-type BALB/c mice. IgE was prepared from hybridoma IGEL b4, because TNP-IgE transgenic mice carry the genes that encode the heavy and light chains of IGEL b4, an anti-TNP-IgE-producing hybridoma (24). The i.v. administration of TNP-IgE caused increased MDC levels (Fig. 1B). MDC production reached a peak level at 24–30 h after IgE injection and returned to a basal level after 48 h (data not shown). TNP-IgE-induced MDC production was almost completely abrogated in FcRγ (−/−) mice, but not in wild-type C57BL/6 mice (Fig. 1B). FcRγ (−/−) mice lack not only the IgE receptor, but also

the IgG receptor. However, it was unlikely that MDC production was mediated by the IgG receptor. The administration of control IgG (MOPC-31c; Sigma-Aldrich) into BALB/c mice did not result in the increased MDC levels (data not shown). Thus, these data suggest that IgE stimulates MDC synthesis through the FcεRI receptor. The contamination of endotoxin in prepared IgE samples was less than 0.005 EU/ml (Endospecy ES-50M kit; Seikagaku). Moreover, the increase in serum MDC levels by IgE injection was also observed even in TLR4-mutant C3H/HeJ mice (238.6 ± 18.39 pg/ml vs 331.2 ± 28.4 pg/ml, control and TNP-IgE-injected mice, respectively, p < 0.05).

MDC production in mast cell-deficient mice

Because mast cells constitute a major population of FcεRI-expressing cells in mice, we postulated that mast cells could be a source of MDC and that TNP-IgE-induced MDC production would be abrogated in mast cell-deficient mice. Unexpectedly, however, the serum levels of MDC were weakly but significantly increased after TNP-IgE injection in mast cell-deficient WBB6F₁-Sl/Sld mice (Fig. 1C). Thus, cells other than mast cells were secreting MDC in response to IgE.

MDC production from bone marrow cells

To further explore the source of MDC, we examined MDC production from bone marrow cells. Freshly isolated bone marrow cells from BALB/c mice were incubated with monomeric
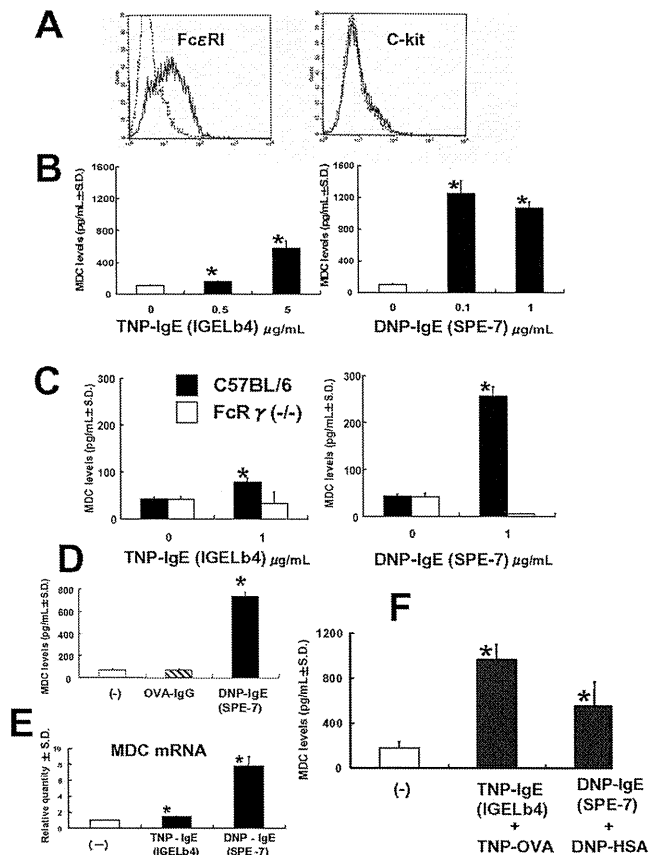
FIGURE 3. MDC production from basophils. *A*, BMBa were prepared as described in *Materials and Methods*. These cells expressed FcεRI but not c-*kit*. Dotted lines indicate negative control staining. *B*, BMBa secreted a large amount of MDC in response to IgE. DNP-IgE appeared to be more potent than TNP-IgE. *C*, Deficiency of the FcRγ-chain resulted in the complete abrogation of MDC production from BMBa. *D*, OVA-IgG (5 μg/ml) was not capable of inducing MDC production in BMBa from BALB/c mice. *E*, Stimulation of BMBa with IgE for 24 h induced a small but significant increase in MDC mRNA synthesis as assessed by real-time PCR analysis. *F*, BMBa were primed with TNP-IgE (0.5 μg/ml) or DNP-IgE (0.5 μg/ml) overnight, followed by stimulation with 20 ng/ml TNP-OVA or DNP-HSA. Results representative of three separate experiments are shown. *, $p < 0.05$.

FIGURE 4. Activation of Lyn and ERK1/2 by IgE or by IgE+Ag in BMBa. *A*, BMBa were stimulated by TNP-IgE (5 μg/ml) or DNP-IgE (SPE-7) (1 μg/ml) for the indicated periods. Cell lysates were analyzed by immunoblotting with the indicated Abs. The exposure time for detecting positive signals for TNP-IgE was much longer than that for DNP-IgE, as the intensities of signals triggered by TNP-IgE were weaker than by DNP-IgE. Therefore, the signal intensities between these two gels are not comparable. *B*, BMBa were incubated overnight with TNP-IgE (0.5 μg/ml) or DNP-IgE (SPE-7) (0.5 μg/ml). They were then stimulated with TNP-OVA or DNP-HSA for the indicated periods. The exposure times for the *left* and *right panels* were the same but much shorter than those of IgE alone (*A*), because the signals triggered by IgE+Ags were strong. The activation of Lyn and ERK1/2 occurred earlier and stronger in IgE+Ags than in IgE alone. Results are representative of three separate experiments.

TNP-IgE for 24 h. MDC was secreted when these cells were stimulated with TNP-IgE in the absence of a specific Ag in vitro (Fig. 2*A*, *left panel*). To determine whether IgE-mediated MDC production was limited to TNP-IgE (IGEL b4), we tested the effect of SPE-7, a type of DNP-specific IgE that is considered highly cytokinergic (17, 19, 20, 22). DNP-IgE (SPE-7) also stimulated MDC secretion from freshly isolated bone marrow cells (Fig. 2*A*, *right panel*). MDC production was not observed in bone marrow cells from FcRγ-chain (−/−) mice (Fig. 2*B*). In addition, depletion of FcεRI (+) cells resulted in the remarkable reduction of TNP-IgE-induced MDC secretion by freshly isolated bone marrow cells (from 49.0 ± 4.60 pg/ml to 9.3 ± 2.59 pg/ml), further confirming the involvement of FcεRI-bearing cells in TNP-IgE-mediated MDC release. Freshly isolated bone marrow cells, even from the mast cell-deficient mice, generated MDC (Fig. 2*C*), which was consistent with increased MDC levels in vivo in these mice (Fig. 1*C*).

Basophils produce MDC in response to IgE

Prior evidence indicates that bone marrow does not contain mature mast cells that express FcεRI (31). Consistent with this evidence, we were unable to detect FcεRI (+)/c-*kit* (+) mast
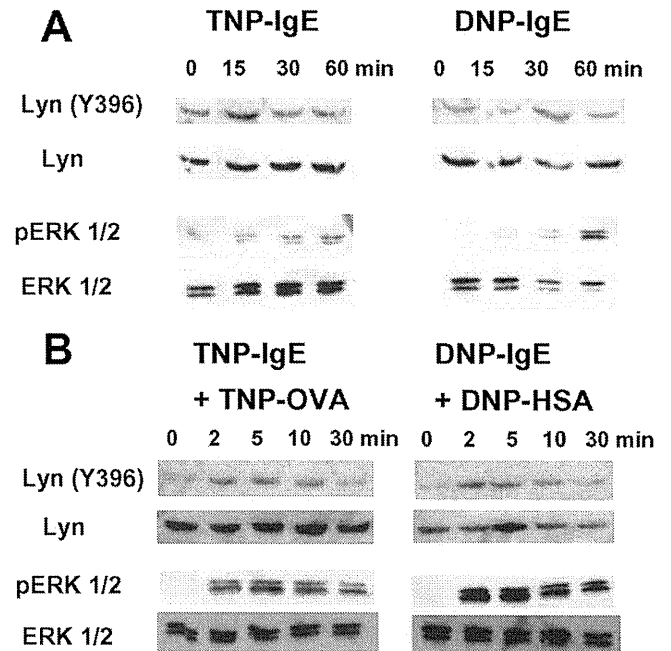
cells in freshly isolated bone marrow cells. There were FcεRI (+)/c-*kit* (−) cells that constituted less than 2% of bone marrow cells in BALB/c mice and even in mast cell-deficient WBB6F₁-Sl/Sld mice (data not shown). Thus, we postulated that MDC secreted from fresh bone marrow cells was derived from another type of FcεRI-expressing cell, i.e., basophils.

Next, we prepared basophils by culturing Lin (−) bone marrow-progenitor cells maintained with IL-3 for 8–10 days followed by the depletion of c-*kit* (+) cells (Fig. 3*A*). As expected, BMBa produced a significant amount of MDC when stimulated with IgE (Fig. 3*B*). An almost complete abrogation of MDC release was observed in FcRγ (−/−)-derived BMBa (Fig. 3*C*). Moreover, OVA-specific IgG was not capable of stimulating BMBa of BALB/c mice to secrete MDC (Fig. 3*D*), again confirming the involvement of FcεRI but not IgG receptors.

Unstimulated BMBa expressed MDC mRNA (data not shown). In addition, IgE stimulation resulted in enhanced MDC mRNA synthesis as assessed by real-time PCR analysis (Fig. 3*E*). The increase in the level of MDC mRNA was detected at 6 h (data not shown) and peaked at 24 h for DNP-IgE, whereas TNP-IgE-induced mRNA synthesis had a small but statistically significant increase that was detected only at 24 h.

Comparison of MDC production from BMBa between IgE and IgE+Ags

The capability of BMBa to secrete MDC via signaling through FcεRI was further confirmed by the remarkable MDC generation
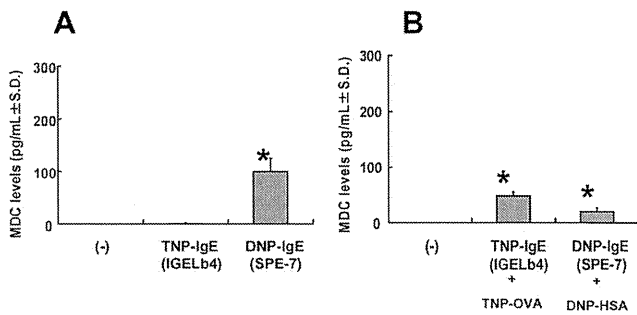
FIGURE 5. MDC production of BMMC. *A*, A small amount of MDC was secreted from BMMC after stimulation with DNP-IgE (1 µg/ml), but MDC was undetectable after treatment with TNP-IgE (5 µg/ml). *B*, TNP-IgE induced comparable levels of MDC production to DNP-IgE when cells were stimulated together with corresponding Ags. Results of three separate experiments are shown. *, $p < 0.05$.

that occurred when BMBa were stimulated with IgE plus the corresponding Ags. Notably, the activation abilities of TNP- and DNP-IgEs were similar when stimulated together with Ags (Fig. 3*F*), while TNP-IgE alone appeared to be less cytokinergic than DNP-IgE alone (Fig. 3*B*).

FcεRI-mediated signaling in mast cells includes several activation events, such as the activation of Lyn, Syk, Btk, and MAPKs (16, 19, 22). In BMBa, both TNP- and DNP-IgE induced phosphorylation of Lyn and ERK 1/2 (Fig. 4*A*). Similarly, IgE+Ags activated these signals in a manner that was faster and/or stronger than the signals induced by IgE alone (Fig. 4*B*).

MDC secretion from mast cells

We next tested whether mast cells were capable of producing MDC in vitro. BMMC were stimulated with TNP- or DNP-IgE. As shown in Fig. 5*A*, DNP-IgE stimulated mast cells to produce MDC, but the protein levels were much lower than those of BMBa (Fig. 3*B*), and the MDC secretion induced by TNP-IgE was undetectable. Despite the low capability of MDC production by IgE, BMMC treated with IgE exhibited significant degranulation as assessed by the β-hexosaminidase assay (31.8 ± 2.72% vs 3.4 ± 0.49% for TNP-IgE and negative control, respectively). Both TNP- and DNP-IgE induced MDC secretion from BMMC when they were stimulated together with their corresponding Ags (Fig. 5*B*). However, the amount of MDC secreted by BMMC was much less than the amount of MDC secreted by BMBa (Fig. 3*F*).
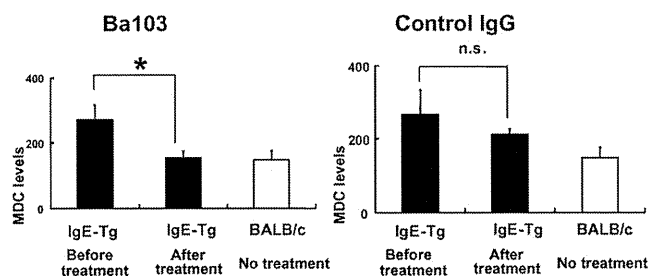

FIGURE 6. Effect of basophil depletion on serum MDC. TNP-IgE transgenic mice were treated with basophil depletion Ab (Ba103). Serum was collected before and 4 days after the treatment. The depletion of basophils resulted in decreased serum MDC levels ($n = 5$). Results of three separate experiments are shown. *, $p < 0.05$.

Depletion of basophils reduces serum MDC in vivo

To confirm that basophils secreted MDC in vivo, basophil depletion Ab (Ba103) (32) or control rat IgG (Sigma-Aldrich) was i.v. injected into IgE transgenic mice (30 µg/mouse), and serum levels of MDC before and after Ab treatment were measured. As expected, the serum levels of MDC decreased after basophil depletion (Fig. 6). The effect was most marked at 4 days after injection and gradually returned to the basal level within two weeks (data not shown). MDC production from basophils in vivo was further confirmed by the results that wild-type BALB/c mice pretreated with Ba103 Ab 4 days before TNP-IgE injection did not exhibit inducible synthesis of serum MDC (data not shown).

Discussion

The TNP-IgE transgenic mice, which were generated by transgenes from a TNP-specific IgE-producing hybridoma (IGEL b4), have persistently high levels of IgE in their sera (~20–40 µg/ml) (24). These mice show no significant abnormalities at birth or during postnatal growth. However, the peritoneal mast cells in IgE transgenic mice express 6 to 8 times higher amounts of FcεRI on their surface than do the peritoneal mast cells from their nontransgenic littermates (24). This is probably due to the IgE-mediated inhibition of FcεRI internalization (33). In the present study, we observed another unique phenotype of TNP-IgE transgenic mice; we found that the serum levels of MDC are persistently higher in the transgenic mice than in wild-type mice. It is likely that the increased level of MDC was mediated by IgE, because the administration of TNP-IgE (IGEL b4) into wild-type mice induced a transient increase in the level of MDC. We identified basophils, but not mast cells, as a major source of IgE-induced MDC.

Recent evidence has revealed that IgE bind to FcεRI results in degranulation and cytokine production without Ag stimulation in mast cells (15–22). In vitro analyses in the present study revealed that BMBa produce a significant amount of MDC in response to IgE in the absence of a specific Ag. This is the first study to demonstrate that monomeric IgE is capable of stimulating basophils to secrete MDC. It was also revealed that TNP-IgE (IGEL b4) is a cytokinergic IgE, although SPE-7 (DNP-IgE) appeared to be more cytokinergic than TNP-IgE.

The precise mechanisms of basophil activation by monomeric IgE have not yet been fully elucidated. However, in studies with mast cells, highly cytokinergic IgEs can induce small clusters of FcεRI aggregates in lipid drafts by reducing repulsion between neighboring FcεRI molecules. This clustering seems to be mediated by interaction between hypervariable regions of IgE molecules (22), as monovalent hapten can inhibit IgE-induced cell activation (17). Thus, signals mediated by IgE alone are basically the same as signals mediated by IgE+Ags in mast cells. In the present study with BMBa, we observed the similarity in the signaling events, such as Lyn and ERK in IgE-induced stimulation vs IgE+Ag-induced stimulation. In addition, intracellular signals triggered by IgE without Ag were weak and slow compared with those triggered by IgE+Ag, as observed in the prior studies of mast cells (19, 22).

It was interesting that the high production of MDC was sustained in the TNP-IgE transgenic mice, while a single exposure of wild-type mice to TNP-IgE induced a transient MDC generation that peaked 24 to 28 h after injection and returned to a basal level after 48 h. Weak signals induced by monomeric IgE might enable blood basophils to continuously synthesize MDC

in vivo, probably in collaboration with increased surface expression of FcεRI (33) and/or prolonged cell survival (17), which are also sustained by IgE binding to its receptors. Alternatively, IgE may also bind to premature bone marrow basophils, which are constitutively generated in the bone marrow, and this binding may result in the persistently increased serum levels of MDC.

In contrast to the remarkable production of MDC by BMBa, BMMC secreted lower amounts of MDC (Fig. 5). Data (not shown) also demonstrated that peritoneal mast cells were even less potent than BMMC in secreting MDC. The underlying mechanisms that are responsible for the extremely different capabilities for MDC production between basophils and mast cells are unclear. We found that c-*kit* (−) basophils and c-*kit* (+) cells appearing in in vitro culture of bone marrow cells with IL-3 for 8 days secreted similar amounts of MDC when stimulated with IgE (data not shown), while c-*kit* (+) mature mast cells cultured for 5 wk did not efficiently secrete MDC. It seemed that bone marrow progenitor cells gradually lost their capability for MDC production during the long-term culture with IL-3. Mast cells might preserve enough ability to produce MDC before completing their maturation. Similarly, basophils in the bone marrow may be more potent than mature basophils in the peripheral blood in regard to their MDC-producing capability. It will be interesting to determine the different capabilities for MDC production in different cell lineages and maturation stages.

The enhancement in serum MDC levels of mast cell-deficient WBB6F$_1$-Sl/Sld mice was not marked compared with wild-type C57BL/6 mice (Fig. 2, *B* and *C*), although the increase was statistically significant. A high number of mast cells reside in the skin, gut, respiratory tract, and other organs, as compared with the number of basophils, which constitute a minimal population in blood leukocytes. Thus, the secretion of MDC from mast cells may not be negligible in vivo despite the low capability for MDC production by mast cells in vitro.

MDC exerts its biological effects though chemokine receptor CCR4 expressed on Th2 cells (3, 4). Because TARC (CCL17) is also a ligand for CCR4, we tested the TARC-producing capability of basophils in another set of experiments. Serum levels of TARC in BALB/c mice (74.0 ± 8.37 pg/ml) increased after TNP-IgE injection (160.5 ± 48.70 pg/ml, $p < 0.05$). In addition, BMBa produced TARC in response to IgE in vitro (0 pg/ml, 39.5 ± 2.38 pg/ml, 514.3 ± 20.29 pg/ml, for medium alone, TNP-IgE, and DNP-IgE, respectively). These data may suggest that IgE stimulates basophils to secrete TARC, leading to the involvement of CCR4 (+) Th2 cell recruitment in synergy with MDC. However, unlike MDC, we could not detect a statistically significant increase in basal serum TARC levels in TNP-IgE transgenic mice compared with wild-type BALB/c mice (data not shown). The reason for this discrepancy is unknown. It might be due to differences in serum half life or in the capability for persistent protein synthesis in basophils between MDC and TARC.

Collectively, the present data reveal that overproduction of IgE induces MDC release from basophils. MDC from basophils may contribute to allergic inflammation, a condition in which IgE is persistently increased.

Disclosures

References

1. Chang, M., J. McNinch, C. Elias, 3rd, C. L. Manthey, D. Grosshans, T. Meng, T. Boone, and D. P. Andrew. 1997. Molecular cloning and functional characterization of a novel CC chemokine, stimulated T cell chemotactic protein (STCP-1) that specifically acts on activated T lymphocytes. *J. Biol. Chem.* 272: 25229–25237.
2. Godiska, R., D. Chantry, C. J. Raport, S. Sozzani, P. Allavena, D. Leviten, A. Mantovani, and P. W. Gray. 1997. Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. *J. Exp. Med.* 185: 1595–1604.
3. Andrew, D. P., M. S. Chang, J. McNinch, S. T. Wathen, M. Rihanek, J. Tseng, J. P. Spellberg, and C. G. Elias, 3rd. 1998. STCP-1 (MDC) CC chemokine acts specifically on chronically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13. *J. Immunol.* 161: 5027–5038.
4. Imai, T., M. Nagira, S. Takagi, M. Kakizaki, M. Nishimura, J. Wang, P. W. Gray, K. Matsushima, and O. Yoshie. 1999. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int. Immunol.* 11: 81–88.
5. Bochner, B. S., S. A. Hudson, H. Q. Xiao, and M. C. Liu. 2003. Release of both CCR4-active and CXCR3-active chemokines during human allergic pulmonary late-phase reactions. *J. Allergy Clin. Immunol.* 112: 930–934.
6. Hartl, D., M. Griese, T. Nicolai, G. Zissel, C. Prell, N. Konstantopoulos, R. Gruber, D. Reinhardt, D. J. Schendel, and S. Krauss-Etschmann. 2005. Pulmonary chemokines and their receptors differentiate children with asthma and chronic cough. *J. Allergy Clin. Immunol.* 115: 728–736.
7. Honda, K., M. Arima, G. Cheng, S. Taki, H. Hirata, F. Eda, F. Fukushima, B. Yamaguchi, M. Hatano, T. Tokuhisa, and T. Fukuda. 2003. Prostaglandin D2 reinforces Th2 type inflammatory responses of airways to low-dose antigen through bronchial expression of macrophage-derived chemokine. *J. Exp. Med.* 198: 533–543.
8. Shimada, Y., K. Takehara, and S. Sato. 2004. Both Th2 and Th1 chemokines (TARC/CCL17, MDC/CCL22, and Mig/CXCL9) are elevated in sera from patients with atopic dermatitis. *J. Dermatol. Sci.* 34: 201–208.
9. Kakinuma, T., K. Nakamura, M. Wakugawa, H. Mitsui, Y. Tada, H. Saeki, H. Torii, M. Komine, A. Asahina, and K. Tamaki. 2002. Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. *Clin. Exp. Immunol.* 127: 270–273.
10. Katayama, I., K. Otoyama, H. Yokozeki, and K. Nishioka. 1996. Effect of mast cell modulators on IgE-mediated murine biphasic cutaneous reactions. *Int. Arch. Allergy Immunol.* 109: 390–397.
11. Sato, E., K. Hirahara, Y. Wada, T. Yoshitomi, T. Azuma, K. Matsuoka, S. Kubo, C. Taya, H. Yonekawa, H. Karasuyama, and A. Shiraishi. 2003. Chronic inflammation of the skin can be induced in IgE transgenic mice by means of a single challenge of multivalent antigen. *J. Allergy Clin. Immunol.* 111: 143–148.
12. Mukai, K., K. Matsuoka, C. Taya, H. Suzuki, H. Yokozeki, K. Nishioka, K. Hirokawa, M. Etori, M. Yamashita, T. Kubota, Y. Minegishi, H. Yonekawa, and H. Karasuyama. 2005. Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity* 23: 191–202.
13. Jouvin, M. H., M. Adamczewski, R. Numerof, O. Letourneur, A. Valle, and J. P. Kinet. 1994. Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. *J. Biol. Chem.* 269: 5918–5925.
14. Kinet, J. P. 1999. The high-affinity IgE receptor (FcεRI): from physiology to pathology. *Annu. Rev. Immunol.* 17: 931–972.
15. Kalesnikoff, J., M. Huber, V. Lam, J. E. Damen, J. Zhang, R. P. Siraganian, and G. Krystal. 2001. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 14: 801–811.
16. Kawakami, T., and S. J. Galli. 2002. Regulation of mast-cell and basophil function and survival by IgE. *Nat. Rev. Immunol.* 2: 773–786.
17. Kitaura, J., J. Song, M. Tsai, K. Asai, M. Maeda-Yamamoto, A. Mocsai, Y. Kawakami, F. T. Liu, C. A. Lowell, B. G. Barisas, S. J. Galli, and T. Kawakami. 2003. Evidence that IgE molecules mediate a spectrum of effects on mast cell survival and activation via aggregation of the FcεRI. *Proc. Natl. Acad. Sci. USA* 100: 12911–12916.
18. Pandey, V., S. Mihara, A. Fensome-Green, S. Bolsover, and S. Cockcroft. 2004. Monomeric IgE stimulates NFAT translocation into the nucleus, a rise in cytosol Ca2+, degranulation, and membrane ruffling in the cultured rat basophilic leukemia-2H3 mast cell line. *J. Immunol.* 172: 4048–4058.
19. Kitaura, J., K. Eto, T. Kinoshita, Y. Kawakami, M. Leitges, C. A. Lowell, and T. Kawakami. 2005. Regulation of highly cytokinergic IgE-induced mast cell adhesion by Src, Syk, Tec, and protein kinase C family kinases. *J. Immunol.* 174: 4495–4504.
20. Kitaura, J., T. Kinoshita, M. Matsumoto, S. Chung, Y. Kawakami, M. Leitges, D. Wu, C. A. Lowell, and T. Kawakami. 2005. IgE- and IgE+Ag-mediated mast cell migration in an autocrine/paracrine fashion. *Blood* 105: 3222–3229.
21. Matsuda, K., A. M. Piliponsky, M. Iikura, S. Nakae, E. W. Wang, S. M. Dutta, T. Kawakami, M. Tsai, and S. J. Galli. 2005. Monomeric IgE enhances human mast cell chemokine production: IL-4 augments and dexamethasone suppresses the response. *J. Allergy Clin. Immunol.* 116: 1357–1363.
22. Kawakami, T., and J. Kitaura. 2005. Mast cell survival and activation by IgE in the absence of antigen: a consideration of the biologic mechanisms and relevance. *J. Immunol.* 175: 4167–4173.

23. Takai, T., M. Li, D. Sylvestre, R. Clynes, and J. V. Ravetch. 1994. FcR γ chain deletion results in pleiotrophic effector cell defects. *Cell* 76: 519–529.

24. Matsuoka, K., C. Taya, S. Kubo, N. Toyama-Sorimachi, F. Kitamura, C. Ra, H. Yonekawa, and H. Karasuyama. 1999. Establishment of antigen-specific IgE transgenic mice to study pathological and immunobiological roles of IgE in vivo. *Int. Immunol.* 11: 987–994.

25. Rudolph, A. K., P. D. Burrows, and M. R. Wabl. 1981. Thirteen hybridomas secreting hapten-specific immunoglobulin E from mice with Iga or Igb heavy chain haplotype. *Eur. J. Immunol.* 11: 527–529.

26. Schneider, E., F. Machavoine, J. M. Pleau, A. F. Bertron, R. L. Thurmond, H. Ohtsu, T. Watanabe, A. H. Schinkel, and M. Dy. 2005. Organic cation transporter 3 modulates murine basophil functions by controlling intracellular histamine levels. *J. Exp. Med.* 202: 387–393.

27. Asai, K., J. Kitaura, Y. Kawakami, N. Yamagata, M. Tsai, D. P. Carbone, F. T. Liu, S. J. Galli, and T. Kawakami. 2001. Regulation of mast cell survival by IgE. *Immunity* 14: 791–800.

28. Oki, T., J. Kitaura, K. Eto, Y. Lu, M. Maeda-Yamamoto, N. Inagaki, H. Nagai, Y. Yamanishi, H. Nakajima, H. Kumagai, and T. Kitamura. 2006. Integrin alphaIIbβ3 induces the adhesion and activation of mast cells through interaction with fibrinogen. *J. Immunol.* 176: 52–60.

29. Zhu, F. G., K. Gomi, and J. S. Marshall. 1998. Short-term and long-term cytokine release by mouse bone marrow mast cells and the differentiated KU-812 cell line are inhibited by brefeldin A. *J. Immunol.* 161: 2541–2551.

30. Schwartz, L. B., K. F. Austen, and S. I. Wasserman. 1979. Immunologic release of β-hexosaminidase and β-glucuronidase from purified rat serosal mast cells. *J. Immunol.* 123: 1445–1450.

31. Jamur, M. C., A. C. Grodzki, E. H. Berenstein, M. M. Hamawy, R. P. Siraganian, and C. Oliver. 2005. Identification and characterization of undifferentiated mast cells in mouse bone marrow. *Blood* 105: 4282–4289.

32. Obata, K., K. Mukai, Y. Tsujimura, K. Ishiwata, Y. Kawano, Y. Minegishi, N. Watanabe, and H. Karasuyama. 2007. Basophils are essential initiators of a novel type of chronic allergic inflammation. *Blood* 110: 913–920.

33. Kubo, S., K. Matsuoka, C. Taya, F. Kitamura, T. Takai, H. Yonekawa, and H. Karasuyama. 2001. Drastic up-regulation of FcεRI on mast cells is induced by IgE binding through stabilization and accumulation of FcεRI on the cell surface. *J. Immunol.* 167: 3427–3434.

Newly discovered roles for basophils: a neglected minority gains new respect

Hajime Karasuyama, Kaori Mukai, Yusuke Tsujimura and Kazushige Obata

Abstract | Basophils are the least common type of granulocyte and they account for less than 1% of peripheral blood leukocytes. Because of this minority status and a phenotype that is similar to mast cells, basophils have often been neglected in immunological studies or considered to have minor, redundant roles in immune responses *in vivo*. However, recent studies have now defined previously unrecognized roles for basophils in both immune regulation and allergic responses, and have shown that basophils and mast cells have distinct roles in immune responses.

Basophils, neutrophils and eosinophils are classified as granulocytes, which are characterized by the presence of lobulated nuclei and secretory granules in the cytoplasm. Basophils normally circulate in the bloodstream and under certain pathological conditions, such as allergic disorders and parasite infections, are recruited to peripheral tissues, albeit in small numbers[1–3]. Basophils are defined by the presence of basophilic granules in the cytoplasm, by the surface expression of the high-affinity Fc receptor for IgE (FcεRI) and by the release of chemical mediators, such as histamine, after stimulation. As these characteristics are shared with mast cells, basophils have often been studied as surrogates of the less-accessible tissue-resident mast cells in human studies despite the apparent differences in their development and life cycle. Basophils complete their maturation in the bone marrow before they enter the peripheral blood, whereas mast cells exit the bone marrow as progenitors and mature in the peripheral tissues, where they reside for the duration of their lifespan. The lifespan of basophils (several days) is much shorter than that of mast cells (weeks or months), and basophils do not proliferate after they mature, unlike mast cells. The detection of basophils is difficult, particularly in mice, owing to their rarity (less than 1% of leukocytes in the peripheral blood, spleen and bone marrow are basophils)

and because mouse basophils have fewer basophilic granules than human basophils[4]; this has sometimes led to the erroneous conclusion that basophils may not exist in mice (reviewed in REF. 5).

The view that basophils are a minor and redundant variant of mast cells in the blood was held for many years, until the early 1990s when it was discovered that basophils are an important source of T helper 2 (T_H2)-type cytokines. Indeed, it is now clear that basophils from humans and mice rapidly secrete larger quantities of interleukin-4 (IL-4) than T_H2 cells in response to various stimuli, including signalling through FcεRI[6–9]. This finding suggested that basophils may be involved in mediating allergic diseases and immunity to pathogens such as helminths. However, further investigation of such potential basophil functions has long been hampered by the lack of suitable animal models, including mice that are deficient only in basophils. Recent studies have overcome this problem by using the *in vivo* transfer of basophils from normal mice to FcεRI-deficient mice or by establishing basophil-deficient mice with the use of basophil-depleting antibodies[10–14] (BOX 1).

Box 1 | New tools for studying the *in vivo* function of basophils

Compared with the extensive research on the roles of mast cells, basophils have not been thoroughly studied. This can be attributed to the paucity of basophil sources and the lack of appropriate analytical tools. Researchers carrying out biochemical and functional analyses of basophils are often faced with the difficulty of collecting them in sufficient numbers, particularly from mice. In addition, there are currently no available cell lines that are relevant to basophils. As far as we are aware, no one has succeeded in establishing mouse basophil-specific monoclonal antibodies that can be used for the identification and purification of basophils. Furthermore, although there are naturally occurring mutant mice that lack mast cells, such as WBB6F1-*Kit*^W/W-v and C57BL/6-*Kit*^W-sh/W-sh mice[35], there are no appropriate animal models for studying the function of basophils — for example, mutant animals that are deficient only in basophils.

Recent studies have successfully established a basophil-deficient state in mice by treating them with basophil-depleting antibodies[11–14]. To deplete basophils, we have used a newly established monoclonal antibody that is specific for the CD200 receptor 3 (CD200R3), known as Ba103 (REFS 11,12,15), and two other groups have used an antibody that is specific for the high-affinity Fc receptor for IgE (FcεRIα), known as MAR-1 (REFS 13,14). Both antibodies specifically bind to basophils and mast cells *ex vivo*. Importantly, when administered *in vivo*, these antibodies preferentially deplete circulating basophils and show minimal effect on tissue-resident mast cells. In addition, MAR-1 binding to FcεRIα does not induce interleukin-4 production by basophils *in vitro*[13]. The use of MAR-1 or Ba103 together with mast-cell-deficient mice can exclude the possible side effects of the antibodies on mast cells. Basophil depletion by Ba103 is mediated by a complement-independent but Fc receptor γ-chain-(FcRγ)-dependent mechanism, which suggests the involvement of FcR-mediated phagocytosis or cytotoxicity[11]. The generation and use of Ba103 and MAR-1 monoclonal antibodies can largely account for the recent considerable progress in basophil research[11–14]. However, we need to further refine the tools for basophil analysis, including the establishment of genetically engineered mice that are deficient only in basophils. The identification of genes that are selectively expressed in basophils should help us to achieve this goal.

For human basophils, the flow-cytometry-based basophil activation test, which involves the use of antibodies that are specific for CD203c and CD63 to identify activated basophils, is proving to be a useful tool for the diagnosis of allergies.
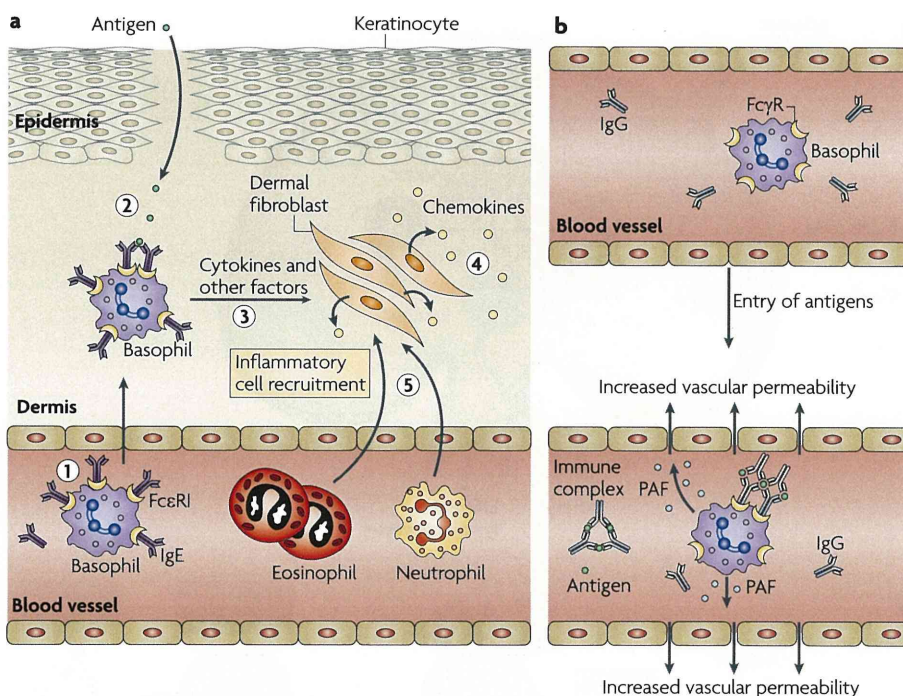

Figure 1 | **Roles for basophils in allergy. a** | Basophils initiate chronic allergic inflammation. In antigen-sensitized animals, antigen-specific IgE antibodies are produced and captured by circulating basophils through the high-affinity Fc receptor for IgE (FcεRI) (**step 1**). When the same antigens enter the skin tissue, IgE-bearing basophils are recruited to the skin lesion, possibly by chemokines released by phagocytic cells that have captured antigen (not shown), and are activated when the antigen binds to the IgE–FcεRI complex (**step 2**). Activated basophils secrete cytokines and other factors (**step 3**) that in turn act on tissue-resident cells, such as fibroblasts, and induce them to produce more chemokines (**step 4**). Inflammatory cells, such as eosinophils and neutrophils, are then recruited to the skin lesion in response to the chemokines that are released by tissue-resident cells (**step 5**), resulting in chronic allergic inflammation. **b** | Basophils induce IgG-mediated systemic anaphylaxis. In allergen-sensitized animals, antigen-specific IgG antibodies are produced and circulate in the blood. When the same antigens enter the blood, antigen–IgG immune complexes are formed and immediately captured by circulating basophils through Fc receptors for IgG (FcγRs) on their surface. Basophils are activated by the immune complexes and release the potent vasoamine platelet-activating factor (PAF), which in turn increases vascular permeability by acting on endothelial cells, thereby leading to systemic anaphylaxis.

As we describe in this Progress article, such studies have defined previously unrecognized roles for basophils in immune regulation and allergic responses that are distinct from those of mast cells. This moves basophils out of obscurity and into the forefront of many aspects of immunological research.

Basophils in allergic reactions

Basophils as initiators of chronic allergy.
Similarly to mast cells, basophils have long been thought to be effector cells in allergic disorders, in which they elicit allergic responses through the release of chemical mediators, such as histamine and leukotriene C4 (REFS 1–3). We have recently shown that basophils have a crucial role in the development of IgE-mediated chronic allergic inflammation in the skin as initiators rather than effectors of the inflammatory

response[10,11] (FIG. 1a). A single subcutaneous injection of multivalent antigen in the ear of mice that had been passively sensitized with antigen-specific IgE induced immediate- and late-phase ear swelling as well as delayed-onset ear swelling with massive eosinophil infiltration[10]. Both the immediate- and late-phase responses were mediated by mast cells as expected, but neither mast cells nor T cells were required for the delayed-onset, IgE-mediated allergic inflammation. The *in vivo* transfer of various cell lineages from wild-type mice to FcεRI-deficient mice that could not mount this response showed that basophils, as defined by cell-surface markers and morphology, were responsible for the induction of the IgE-mediated chronic allergic inflammation even though they accounted for only ~2% of the infiltrates in the skin lesions[10].

This finding was confirmed by studies in our laboratory in which the newly developed monoclonal antibody Ba103, which is specific for CD200 receptor 3 (CD200R3; a CD200 receptor-like glycoprotein), was used to deplete basophils in mice[15]. Basophil depletion during progression of the IgE-mediated dermatitis had a therapeutic effect on inflammation and resulted in a marked decrease in the number of infiltrating eosinophils and neutrophils, together with the elimination of basophils from the skin lesions[11]. This would not be expected if basophils, eosinophils and neutrophils were recruited independently to the skin lesions. A possibility that arose from our preliminary experiments was that antigen-activated basophils secrete soluble factors that stimulate tissue-resident non-haematopoietic cells, such as fibroblasts, to produce various chemokines that in turn recruit inflammatory cells, including eosinophils and neutrophils, to the skin lesions (FIG. 1a). These results suggested that basophils have an important and non-redundant role in chronic allergic inflammation as initiators rather than effectors of the inflammatory response. The finding that the small number of basophils in the skin can initiate severe chronic allergic inflammation in this mouse model implies that we need to reassess the role of basophils in human allergic disorders, such as atopic dermatitis and asthma, in which tissue infiltration by a small number of basophils is often observed[16,17].

Basophils in IgG-mediated anaphylaxis.
Anaphylaxis is a rapid-onset, life-threatening allergic reaction[18,19]. It is well known that mast cells and IgE are crucially involved in systemic anaphylaxis[20]. In allergen-sensitized individuals, allergen-specific IgE antibodies are produced and bind to tissue-resident mast cells through FcεRI. Following re-exposure to the same allergen, allergen-mediated cross-linking of IgE-bound FcεRI activates mast cells to release anaphylaxis-inducing chemical mediators such as histamine. These chemical mediators increase vascular permeability by acting on endothelial cells, thereby leading to anaphylactic shock. However, this scenario cannot explain all cases of anaphylaxis; mice deficient for either mast cells or IgE still develop systemic anaphylaxis, which indicates that an alternative pathway (or pathways) exists[20,21].

In addition to IgE, IgG — particularly the IgG1 subclass — has been shown to elicit anaphylaxis in mice[22,23], and cells other than mast cells, including macrophages, were

—113—