Ⅳ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

英語論文

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Cheng SW, Kobayashi M, Tanikawa A, Kinoshita-Kuroda K, Amagai M, Nishikawa T.	Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmoglein 1 and 3.	**Br J Dermatol**	147	261-265	2002
Amagai M, Yamaguchi T, Hanakawa Y, Nishifuji K, Sugai M, and Stanley JR.	Staphylococcal Exfoliative Toxin B Specifically Cleaves Desmoglein 1.	**J Invest Dermatol**	118	845-850	2002
Ohyama M, Amagai M, Tsunoda K, Ota T, Koyasu S, Umezawa A, Hata J, and Nishikawa T.	Immunologic and histopathologic characterization of active disease mouse model for pemphigus vulgaris.	**J Invest Dermatol**	118	199-204	2002
Hahn-Ristic K, Rzany B, Amagai M, Brocker E, and D. Z.	Increased incidence of pemphigus vulgaris in Southern Europeans living in Germany compared to native Germans.	**J Eur Acad Dermatol Venereol**	16	68-71	2002
Hanakawa Y, Schechter NM, Lin C, Garza L, Li H, Yamaguchi T, Fubada Y, Nishifuji K, Sugai M, Amagai M, and Stanley JR.	Molecular mechanisms of blister formation in bullous impegito and staphylococcal scalded skin syndrome.	**J Clin Invest**	110	53-60	2002
Yamaguchi T, Nishifuji K, Sasaki M, Fudaba Y, Aepfelbacher M, Takata T, Ohara M, Komatsuzawa H, Amagai M, and Sugai M.	Identification of *Staphylococcus aureus etd* pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B.	**Infect Immun**	70	5835-5845	2002
Kobayashi M, Amagai M, Kuroda-Kinoshita K, Hashimoto T, Shirakata Y, Hashimoto K, and Nishikawa T.	BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid.	**J Dermatol Sci**	30	224-232	2002
Shimizu A, Ishiko A, Ota T, Tsunoda K, Koyasu S, Amagai M, and Nishikawa T.	The ultrastructural changes of mice actively producing antibodies to desmoglein3 parallel those in patients with pemphigus vulgaris.	**Arch Dermatol Res**	294	318-323	2002
Hanakawa Y, Amagai M, Shirakata Y, Yahata Y, Tokumaru S, Yamasaki K, Tohyama M, Sayama K, and Hashimoto K.	Differential effects of desmoglein 1 and desmoglein 3 on desmosome formation.	**J Invest Dermatol**	119	1224-1230	2002
Tsunoda K, Ota T, Suzuki H, Ohyama M, Nagai T, Nishikawa T, Amagai M, and Koyasu S.	Pathogenic autoantibody production requires loss of tolerance against desmoglein 3 in both T and B cells in experimental pemphigus vulgaris.	**Eur J Immunol**	32	627-633	2002
Matsui M, Motomura D, Fujikawa T, Jiang J, Takahashi S, Manabe T, Taketo MM	Mice lacking M2 and M3 muscarinic acetylcholine receptors are devoid of smooth muscle contractions but still viable.	**J Neurosci**	22	10627-10632	2002
Fukao, T., Yamada, T., Tanabe, M., Terauchi, Y., Ota, T., Takayama, T., Asano, T., Takeuchi, T., Kadowaki, T., Hata, J. and Koyasu S.	Selective loss of gastrointestinal mast cells and impaired immunity in PI3K-deficient mice.	**Nat. Immunol.**	3	295-304	2002
Agematsu, K., Futatani, T., Hokibara, S., Kobayashi, N., Takamoto, M., Tsukada, S., Suzuki, H., Koyasu S., Miyawaki, T., Sugane, K., Komiyama, A. and Ochs, H. D.	Absence of memory B cells in patients with common variable immunodeficiency.	**Clin. Immunol.**	103	34-42	2002

Fukao, T., Tanabe, M., Terauchi, Y., Ota, T., Matsuda, S., Asano, T., Kadowaki, T., Takeuchi, T. and Koyasu S.	PI3K-mediated negative feedback regulation of IL-12 production in dendritic cells.	**Nat. Immunol.**	3	875-881	2002
Murata, Y., Ohteki, T., Koyasu S. and Hamuro, J.	IFN-g production by macrophages and dendritic cells dictated by intracellular thiol redox status.	**Eur. J. Immunol.**	32	2866-2873	2002
Yoshida K, Arai T, Kaburaki J, Ikeda Y, Kawakami Y, Kuwana M.	Restricted T cell receptor b-chain usage by T cells autoreactive to b_2-glycoprotein I in patients with antiphospholipid syndrome.	**Blood**	99	2499-2504	2002
Kuwana M, Okazaki Y, Kaburaki J, Kawakami Y, Ikeda Y.	Spleen is a primary site for activation of platelet-reactive T and B cells in patients with immune thrombocytopenic purpura.	**J Immunol**	168	3675-3682	2002
Kuwana M.	Induction of anergic and regulatory T cells by plasmacytoid dendritic cells and other dendritic cell subsets.	**Hum Immunol**	63	1156-1163	2002
Ohyama M, Ota T, Aoki M, Tsunoda K, Harada R, Koyasu S, Nishikawa T, and Amagai M.	Suppression of the immune response against exogenous desmoglein 3 in desmoglein 3 knockout mice: An implication for gene therapy.	**J Invest Dermatol**	120	610-615	2003
Karlhofer FM, Hashimoto T, Slupetzky K, Kiss M, Liu Y, Amagai M, Pieczkowski F, Foedinger D, Kirnbauer R, and Stingl G.	230 kDa and 190 kDa proteins in addition to desmoglein 1 as immunological targets in a subset of pemphigus foliaceus with a combined cell surface and basement membrane zone immune staining pattern.	**Exp Dermatol**	12	646-654	2003
Tsunoda K, Ota T, Aoki M, Yamada T, Nagai T, Nakagawa T, Koyasu S, Nishikawa T, and Amagai M.	Induction of pemphigus phenotype by a mouse monoclonal antibody against the amino-terminal adhesive interface of desmoglein 3.	**J Immunol**	170	2170-2178	2003
Hisamatsu Y, Abreu Velez AM, Amagai M, Ogawa MM, Kanzaki T, and Hashimoto T.	Comparative study of autoantigen profile between Colombian and Brazilian types of endemic pemphigus foliaceus by various biochemical and molecular biological techniques.	**J Dermatol Sci**	32	33-41	2003
Nishifuji K, Amagai M, Ota T, Nishikawa T, and Iwasaki T.	Cloning of canine desmoglein 3 and immunoreactivity of serum antibodies in human and canine pemphigus vulgaris with its extracellular domains.	**J Dermatol Sci**	32	181-191	2003
Ota T, Amagai M, Watanabe M, and Nishikawa T.	No involvement of IgG autoantibodies against extracellular domains of desmoglein 2 in paraneoplastic pemphigus or inflammatory bowel diseases.	**J Dermatol Sci**	32	137-141	2003
Nishifuji K, Amagai M, Nishikawa T, and Iwasaki T.	Production of recombinant extracellular domains of canine desmoglein 1 (Dsg1) by baculovirus expression.	**Vet Immunol Immunopathol**	95	177-182	2003
Futei Y, Amagai M, Hashimoto T, and Nishikawa T.	Conformational epitope mapping and IgG subclass distribution of desmoglein 3 in paraneoplastic pemphigus.	**J Am Acad Dermatol**	49	1023-1028	2003

Hacker-Foegen MK, Janson M, Amagai M, Fairley JA, and Lin MS.	Pathogenicity and epitope characteristics of anti-desmoglein-1 from pemphigus foliaceus patients expressing only IgG1 autoantibodies.	**J Invest Dermatol**	121	1373-1378	2003
Aihara T, Fujishita T, Kanatani K, Furutani K, Nakamura E, Taketo MM, Matsui M, Chen D, Okabe S.	Impaired Gastric Secretion and Lack of Trophic Responses to Hypergastrinemia in M_3 Muscarinic Receptor-knockout Mice.	**Gastroenterology**	125	1774-1784	2003
Ohno-Shosaku T, Matsui M, Fukudome Y, Shosaku J, Tsubokawa H, Taketo MM, Manabe T, Kano M.	Postsynaptic M_1 and M_3 receptors are responsible for the muscarinic enhancement of retrograde endocannabinoid signaling in the hippocampus.	**Eur J Neurosci**	18	109-16	2003
Karasawa H, Taketo MM, Matsui M.	Loss of anti-cataleptic effect of scopolamine in mice lacking muscarinic acetylcholine receptor subtype 4.	**Eur J Pharmacol**	468	15-19	2003
Matsui M, Griffin MT, Shehnaz D, Taketo MM, Ehlert FJ.	Increased Relaxant Action of Forskolin and Isoproterenol against Muscarinic Agonist-Induced Contractions in Smooth Muscle from M_2 Receptor Knockout Mice.	**J Pharmacol Exp Ther**	305	106-113	2003
Suzuki, H., Matsuda, S., Terauchi, Y., Fujiwara, M., Ohteki, T., Asano, T., Behrens, T. W., Kouro, T., Takatsu, K., Kadowaki, T. and Koyasu S.	PI3K and Btk differentially regulate B cell antigen receptor mediated signal transduction.	**Nat. Immunol.**	4	280-286	2003
Suzue, K., Asai, T., Takeuchi, T. and Koyasu S.	*In vivo* role of IFN-g produced by antigen presenting cells in early host defense against intracellular pathogens.	**Eur. J. Immunol.**	33	2666-2675	2003
Yuki N, Saperstein DS.	Axonal Guillain-Barré syndrome subtypes: do we need more splitting?	**Neurology**	61	598-599	2003
Koga M, Yuki N, Tsukada Y, Hirata K, Matsumoto Y.	CDR3 spectratyping analysis of the T cell receptor repertoire in Guillain-Barré and Fisher syndromes.	**J Neuroimmunol**	141	112-117	2003
Susuki K, Nishimoto Y, Yamada M, Baba M, Ueda S, Hirata K, Yuki N.	Acute motor axonal neuropathy rabbit model: immune attack on nerve root axons.	**Ann Neurol**	54	393-388	2003
Odaka M, Yuki N, Yamada M, Koga M, Takemi T, Hirata K, Kuwabara S.	Bickerstaff's brainstem encephalitis: clinical features of 62 cases and a subgroup associated with Guillain-Barré syndrome.	**Brain**	126	2279-2290	2003
Odaka M, Yuki N, Hirata K.	Patients with chronic inflammatory demyelinating polyneuropathy initially diagnosed as Guillain-Barré syndrome.	**J Neurol**	250	913-916	2003
Ikuta N, Fukusako T, Yuki N, Morimatsu M, Koga M.	Acute oropharyngeal palsy associated with anti-GM1b IgG antibody.	**J Neurol**	250	881-882	2003
Sekiguchi K, Susuki K, Funakawa I, Jinnai K, Yuki N.	Cerebral white matter lesions in acute motor axonal neuropathy.	**Neurology**	61	272-273	2003

Susuki K, Johkura K, Yuki N, Kuroiwa Y.	Clinical deterioration in Bickerstaff's brainstem encephalitis caused by overlapping Guillain-Barré syndrome.	**J Neurol**	211	89-92	2003
Odaka M, Yuki N, Kokubun N, Hirata K, Kuwabara S.	Axonal Guillain-Barré syndrome associated with axonal Charcot-Marie-Tooth disease.	**J Neurol Sci**	211	93-97	2003
Ogawara K, Kuwabara S, Koga M, Mori M, Yuki N, Hattori T.	Anti-GM1b IgG antibody is associated with acute motor axonal neuropathy and *Campylobacter jejuni* infection.	**J Neurol Sci**	210	41-45	2003
Odaka M, Koga M, Yuki N, Susuki K, Hirata K.	Longitudinal changes of anti-ganglioside antibodies before and after Guillain-Barré syndrome onset subsequent to Campylobacter jejuni enteritis.	**J Neurol Sci**	210	99-103	2003
Koga M, Yuki N, Hirata K, Morimatsu M, Mori M, Kuwabara S.	Anti-GM1 antibody IgG subclass: a clinical recovery predictor in Guillain-Barré syndrome.	**Neurology**	60	1514-1518	2003
Odaka M, Yuki N.	Antibodies to GM2 ganglioside in neurological disorders.	**Intern Med**	42	220-221	2003
Kuwana M, Kawakami Y, Ikeda Y.	Suppression of autoreactive T-cell response to glycoprotein IIb/IIIa by blockade of CD40/CD154 interaction: implications for treatment of immune thrombocytopenic purpura.	**Blood**	101	621-623	2003
Suzuki S, Tanaka K, Yasuoka H, Fukuuchi Y, Kawakami Y, Kuwana M.	Autoreactive T cells to the $P3A^{+}$ isoform of AChR a subunit in myasthenia gravis.	**J Neuroimmunol**	137	177-186	2003
Kuwana M.	Autoreactive $CD4^{+}$ T cells to b_2-glycoprotein I in patients with antiphospholipid syndrome.	**Autoimmun Rev**	2	192-198	2003
Nomura S, Kuwana M, Ikeda Y.	Induction of T-cell tolerance in a patient with idiopathic thrombocytopenic purpura by single injection of humanized monoclonal antibody to CD40 ligand.	**Autoimmunity**	36	317-319	2003
Araki, M., T. Kondo, J.E. Gumperz, M.B. Brenner, S. Miyake and T. Yamamura	Th2 bias of $CD4^{+}$ NKT cells derived form multiple sclerosis in remission.	**Int. Immunol.**	15	279-288	2003
Miyamoto, K., S. Miyake, M. Schachner, and T. Yamamura	Heterozygous null mutation of myelin P0 protein enhances susceptibility to autoimmune neuritis targeting P0 peptide.	**Eur. J. Immunol.**	33	656-665	2003
Koike, F., J-i. Satoh, T. Kondo, S. Miyake, T. Yamamoto, M. Kawai, S. Kikuchi, K. Nomura, K. Yokoyama, K. Ota, T. Kanda, T. Fukazawa, and T. Yamamura.	Microarray analysis identifies IFNb-regulated genes in multiple sclerosis.	**J. Neuroimunol.**	139	109-118	2003
Nakamura, T., K.-H. Sonoda, D.E. Faunce, J. Gumperz, T. Yamamura., S. Miyake, J. Stein-Streilein.	$CD4^{+}$ NKT cells, but not conventional $CD4^{+}$ T cells, are required to generate efferent $CD8^{+}$ T regulatory cells following antigen inoculation in an immune-privileged site.	**J. Immunol.**	171	1266-1271	2003
Bedoui, S., S. Miyake, Y. Lin, K. Miyamoto, S. Oki, N. Kawamura, A. Beck-Sickinger, S. von Hoersten, and T. Yamamura.	Neuropeptide Y (NPY) suppresses experimental autoimmune encephalomyelitis: NPY Y_1 receptor-specific inhibition of autoreactive Th1 responses in vivo.	**J. Immunol.**	171	3451-3458	2003

Stanic, A.K., R. Shashidharamurthy, J.S. Bezradica, N. Matsuki, Y. Yoshimura, S. Miyake, E.Y. Choi, T.D. Schell, L. Van Kaer, S.S. Tevethia, D.C. Roopenian, T. Yamamura and S. Joyce.	Another view of T cell antigen recoginition: Co-operative engagement of glycolipid antigens by Va14Ja18 natural TCR.	**J. Immunol.**	171	4539-4551	2003
Hanakawa Y, Schechter NM, Lin C, Nishifuji K, Amagai M, and Stanley JR.	Enzymatic and molecular characteristics of the efficiency and specificity of exfoliative toxin cleavage of desmoglein 1.	**J Biol Chem**	279	5268-5277	2004
Shimizu A, Ishiko A, Ota T, Tsunoda K, Amagai M, and Nishikawa T.	IgG binds to desmoglein 3 in desmosomes and causes a desmosomal split without keratin retraction in a pemphigus mouse model.	**J Invest Dermatol**	122	1145-1153	2004
Aoki-Ota M, Tsunoda K, Ota T, Iwasaki T, Koyasu S, Amagai M, and Nishikawa T.	A mouse model of pemphigus vulgaris by adoptive transfer of naive splenocytes from desmoglein 3 knockout mice.	**Br J Dermatol**	151	346-354	2004
Payne AS, Hanakawa Y, Amagai M, and Stanley JR.	Desmosomes and diseases: Pemphigus and bullous impetigo.	**Curr Opin Cell Biol**	16	536-543	2004
Ota T, Aoki-Ota M, Tsunoda K, Simoda K, Nishikawa T, Amagai M, and Koyasu S.	Auto-reactive B cells against peripheral antigen, desmoglein 3, escape from tolerance mechanism.	**Int Immunol**	16	1487-1495	2004
Anzai H, Fujii Y, Nishifuji K, Aoki-Ota M, Ota T, Amagai M, and Nishikawa T.	Conformational epitope mapping of antibodies against desmoglein 3 in experimental murine pemphigus vulgaris.	**J Dermatol Sci**	35	133-142	2004
Hisamatsu Y, Amagai M, Garrod DR, Kanzaki T, and Hashimoto T.	The detection of IgG and IgA autoantibodies to desmocollins 1-3 by enzyme-linked immunosorbent assays using baculovirus-expressed proteins, in atypical pemphigus but not in typical pemphigus.	**Br J Dermatol**	151	73-83	2004
Takahashi H, Anzai H, Suzuki Y, Tanikawa A, Amagai M, Nishikawa T	Parallel fluctuation of anti-desmoglein 3 and anti-BP180 autoantibody titres in a patient with bullous pemphigoid.	**Clin Exp Dermatol**	29	608-611	2004
Nagasaka T, Nishifuji K, Ota T, Whittock NV, and Amagai M.	Defining the pathogenic involvement of desmoglein 4 in pemphigus and staphylococcal scalded skin syndrome.	**J Clin Invest**	114	1484-1492	2004
Niizeki H, Kumagai S, Kanagawa S, Amagai M, Yamashina Y, Asada H, Nishikawa T, and Miyagawa S.	Exclusion of the TAP1 and TAP2 genes within the HLA class II region as candidate susceptibility genes to pemphigus in the Japanese population.	**J Dermatol Sci**	36	122-124	2004
Igawa Y, Zhang X, Nishizawa O, Umeda M, Iwata A, Taketo MM, Manabe T, Matsui M, Andersson KE.	Cystometric findings in mice lacking muscarinic M_2 or M_3 receptors.	**J Urol.**	172	2460-2464	2004
Nakamura T, Matsui M, Uchida K, Futatsugi A, Kusakawa S, Matsumoto N, Nakamura K, Manabe T, Taketo MM, Mikoshiba K.	M_3 muscarinic acetylcholine receptor plays critical role in parasympathetic control of salivation in mice.	**J Physiol.** (London).	558	561-575	2004
Fukudome Y, Ohno-Shosaku T, Matsui M, Omori Y, Fukaya M, Taketo MM, Watanabe M, Manabe T, Kano M.	Two distinct classes of muscarinic action on hippocampal inhibitory synapses: M_2-mediated direct suppression and M_1/M_3-mediated indirect suppression through endocannabinoid signaling.	**Eur J Neurosci.**	19	2682-2692	2004

Matsui M, Yamada S, Oki T, Manabe T, Taketo MM, Ehlert FJ.	Functional analysis of muscarinic acetylcholine receptors using knockout mice (invited review).	**Life Sci.**	75	2971-2981	2004
Zawalich WS, Zawalich KC, Tesz GJ, Taketo MM, Sterpka J, Philbrick W, Matsui M.	Effects of Muscarinic Receptor Type 3 Knockout on Mouse Islet Secretory Responses.	**Biochem Biophys Res Commun**	315	872-876	2004
Griffin MT, Matsui M, Shehnaz D, Ansari KZ, Taketo MM, Manabe T, Ehlert FJ.	Muscarinic agonist-mediated heterologous desensitization in isolated ileum requires activation of both muscarinic M_2 and M_3 receptors.	**J Pharmacol Exp Ther**	308	339-49	2004
Matsuda, S., Miwa, Y., Hirata, Y., Minowa, A., Tanaka, J., Nishida, E. and Koyasu S.	Negative feedback loop in T cell activation through MAPK-catalyzed threonine phosphorylation of LAT.	**EMBO J.**	23	2577–2585	2004
Godschalk PCR, Heikema AP, Gilbert M, Komagamine T, Ang CW, Glerum J, Brochu D, Li J, Yuki N, Jacobs BC, van Belkum A, Endtz HPh.	The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in the Guillain-Barré syndrome.	**J Clin Invest**	114	1659-1665	2004
Yuki N, Susuki K, Koga M, Nishimoto Y, Odaka M, Hirata K, Taguchi K, Miyatake T, Furukawa K, Kobata T, Yamada M.	Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barré syndrome.	**Proc Natl Acad Sci U S A**	101	11404-11409	2004
Susuki K, Nishimoto Y, Koga M, Nagashima T, Mori I, Hirata K, Yuki N.	Various immunization protocols for an acute motor axonal neuropathy rabbit model compared.	**Neurosci Lett**	368	63-67	2004
Kuwabara S, Ogawara K, Misawa S, Koga M, Mori M, Hiraga A, Kanesaka T, Hattori T, Yuki N.	Does *Campylobacter jejuni* infection elicit "demyelinating" Guillain-Barré syndrome?	**Neurology**	63	529-533	2004
Nishimoto Y, Koga M, Kamijo M, Hirata K, Yuki N.	Immunoglobulin improves a model of acute motor axonal neuropathy by preventing axonal degeneration.	**Neurology**	62	1939-1944	2004
Nagashima T, Koga M, Odaka M, Hirata K, Yuki N.	Clinical correlates of serum anti-GT1a IgG antibodies.	**J Neurol Sci**	219	139-45	2004
Susuki K, Yuki N.	Effect of methylprednisolone in patients with Guillain-Barré syndrome.	**Lancet**	363	1236-1237	2004
Susuki K, Odaka M, Mori M, Hirata K, Yuki N.	Acute motor axonal neuropathy after *Mycoplasma* infection: evidence of molecular mimicry.	**Neurology**	62	949-956	2004
Nishimoto Y, Odaka M, Hirata K, Yuki N.	Usefulness of anti-GQ1b IgG antibody testing in Fisher syndrome compared with cerebrospinal fluid examination.	**.J Neuroimmunol**	148	200-205	2004
Galassi G, Susuki K, Quaglino D, Yuki N.	Post-infectious acute ataxia and facial diplegia associated with anti-GD1a IgG antibody.	**Eur J Neurol**	11	790-791	2004
Odaka M, Koga M, Yuki N, Susuki K, Hirata K.	Longitudinal changes of anti-ganglioside antibodies before and after Guillain-Barré syndrome onset subsequent to *Campylobacter jejuni* enteritis.	**Review Series Pediatrics**	2	22-23	2004
Odaka M, Yuki N, Tatsumoto M, Tateno M, Hirata K.	Ataxic Guillain-Barré syndrome associated with anti-GM1b and anti-GalNAc-GD1a antibodies.	**J Neurol**	251	24-29	2004
Mori I, Koga M, Hirata K, Yuki N.	Hand weakness onset Guillain-Barré syndrome.	**J Neurol Neurosurg Psychiatry**	75	169-170	2004

Pan CL, Shun CT, Susuki K, Yuki N, Hsieh ST.	Pharyngeal-brachial palsy after cytomegalovirus colitis.	**Neurology**	62	153-154	2004
Matsuo M, Odaka M, Koga M, Tsuchiya K, Hamasaki Y, Yuki N.	Bickerstaff's brainstem encephalitis associated with IgM antibodies to GM1b and GalNAc-GD1a.	**J Neurol Sci**	217	225-228	2004
Kuwana M, Nomura S, Fujimura K, Nagasawa T, Muto Y, Kurata Y, Tanaka S, Ikeda Y.	The effect of a single injection of humanized anti-CD154 monoclonal antibody on the platelet-specific autoimmune response in patients with immune thrombocytopenic purpura.	**Blood**	103	1229-1236	2004
Kuwana M.	b_2-glycoprotein I: antiphospholipid syndrome and T-cell reactivity.	**Thromb Res**	114	347-355	2004
Yasuoka H, Okazaki Y, Kawakami Y, Hirakata M, Inoko H, Ikeda Y, Kuwana M.	Autoreactive $CD8^+$ cytotoxic T lymphocytes to major histocompatibility complex class I chain-related molecule A in patients with Behçet's disease.	**Arthritis Rheum**	50	3658-3662	2004
Chiba, A., S. Oki, K. Miyamoto, H. Hashimoto, T. Yamamura, and S. Miyake.	Natural killer T-cell activation by OCH, a sphingosine truncated analogue of a-galactosylceramide, prevents collagen-induced arthritis.	**Arthr. Rheumat.**	50	305-313	2004
Illes, Zs., M. Shimamura, J. Newcombe, N. Oka, and T. Yamamura.	Accumulation of Va7.2Ja33 invariant T cells in autoimmune inflammatory lesions of the nervous system.	**Int. Immunol.**	16	223-230	2004
Oki, S., A. Chiba, T. Yamamura and S. Miyake.	The clinical implication and molecular mechanism of preferential IL-4 production by modified glycolipid-stimulated NKT cells.	**J. Clin. Invest.**	113	1631-1640	2004
Satoh, J-i., T. Yamamura, and K. Arima.	The 14-3-3 protein e isoform, expressed in reactive astrocytes in demyelinating lesions of multiple sclerosis, binds to vimentin and glial fibrillary acidic protein in cultured human astrocytes.	**Am. J. Pathol.**	165	577-592	2004
Rosen, D.B., M. Araki, J.A. Hamerman, T. Chen, T. Yamamura and L.L. Lanier.	A structural basis for the association of DAP12 with mouse, but not human, NKG2D.	**J. Immunol.**	173	2470-2478	2004
Takahashi, K., T. Aranami, M. Endoh, S. Miyake, and T. Yamamura.	The regulatory role of natural killer cells in multiple sclerosis.	**Brain**	127	1917-1927	2004
Nakai, Y., K. Iwabuchi, S. Fujii, N. Ishimori, N. Dashtsoodol, K. Watano, T. Mishima, C. Iwabuchi, S. Tanaka, J.S. Bezbradica, T. Nakayama, M. Taniguchi, S. Miyake, T. Yamamura, A. Kitabatake, S. Joyce, L. Van Kaer, and K. Onoe.	Natural killer T cells accelerate atherogenesis in mice.	**Blood**	104	2051-2059	2004
Mizuno, M., M. Masumura, C. Tomi, A. Chiba, S. Oki, T. Yamamura and S. Miyake.	Synthetic glycolipid OCH prevents insulitis and diabetes in NOD mice.	**J. Autoimmun.**	23	293-300	2004
Hashimoto, D., S. Asakura, S. Miyake, T. Yamamura, L. Van Kaer, C. Liu, M. Tanimoto, and T. Teshima.	Stimulation of host natural killer T cells by synthetic glycolipid regulates acute graft-versus-host disease by inducing Th2 polarization of donor T cells.	**J. Immunol.**	174	551-556	2005

Ueno, Y., S. Tanaka, M. Sumii, S. Miyake, S. Tazuma, M. Taniguchi, T. Yamamura and K. Chayama.	Single dose of OCH improves mucosal T helper type 1/T helper type 2 cytokine balance and prevents experimental colitis in the presence of Va14 natural killer T cells in mice.	**Inflamm. Bowel Dis.**	11	35-41	2005
Yu, K.O.A., J.S. Im, A. Molano, Y. Dutronc, P.A. Illarionov, C. Forestier, N. Fujiwara, I. Arias, S. Miyake, T. Yamamura, Y-T. Chang, G.S. Besra, and S.A. Porcelli.	Modulation of CD1d-restrotced NKT cell responses by using N-acyl variants of a-galactosylceramides.	**Proc Natl Acad Sci U S A**	102	3383-3388	2005
Satoh, J-i., M. Nakanishi, F. Koike, S. Miyake, T. Yamamoto, M. Kawai, S. Kukuchi, K. Nomura, K. Yokoyama, K. Ota, T. Kanda, T. Fukazawa and T. Yamamura.	Microarray analysis identifies an abberant expression of apoptosis and DNA damage-regulatory genes in multiple sclerosis.	**Neurobiol. Dis.**	in press		
Ota, T., K. Takeda, H. Akiba, Y. Hayakawa, K. Ogasawara, Y. Ikarashi, S. Miyake, H. Wakasugi, T. Yamamura, M. Kronenberg, D.H. Raulet, K. Kinoshita, H. Yagita, M.J. Smyth, and K. Okumura.	IFN-g-mediated negative feedback regulation of NKT cell function by CD94/NKG2.	**Blood**	in press		
Chiba, A., S. Kaieda, S. Oki, T. Yamamura and S. Miyake.	The involvement of Va14 NKT cells in the pathogenesis of murine models of arthritis.	**Arthr. Rheumat.**	in press		
Kuwana M, Ikeda Y	The role of autoreactive T cells in the pathogenesis of ITP.	**Int J Hematol**	in press		
Kuwana M, Matsuura E, Kobayashi K, Okazaki Y, Kaburaki K, Ikeda Y, Kawakami Y.	Binding of b_2-glycoprotein I to anionic phospholipids facilitates processing and presentation of a cryptic epitope that activates pathogenic autoreactive T cells.	**Blood**	in press		
Takeuchi T, Fujinami K, Goto H, Fujita A, Taketo MM, Manabe T, Matsui M, Hata F.	Roles of M2 and M4 muscarinic receptors in regulating acetylcholine release from myenteric neurons of mouse ileum.	**J Neurophysiol.**	in press		
Ehlert FJ, Griffin MT, Abe DM, Vo TH, Taketo MM, Manabe T, Matsui M.	The M_2 muscarinic receptor mediates contraction through indirect mechanisms in mouse urinary bladder.	**J Pharmacol Exp Ther.**	in press		
Oki T, Takagi Y, Inagaki S, Taketo MM, Manabe T, Matsui M, Yamada S.	Quantitative analysis of binding parameters 6of [^{3}H]N-methylscopolamine in central nervous system of muscarinic acetylcholine receptor knockout mice.	**Brain Res Mol Brain Res.**	in press		

日本語論文

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
天谷雅行	天疱瘡とデスモグレイン.	**Medical Technology**	30	762-763	2002
天谷雅行, 西川武二	天疱瘡と類天疱瘡.	**自己免疫レポート**	27	1-16	2002
天谷雅行	自己免疫性水疱症の最新知見.	**日本臨床免疫学会雑誌**	25	28-31	2002
天谷雅行	ブドウ球菌性熱傷様皮膚症候群(SSSS)におけるデスモグレイン１の関与.	**臨床皮膚科**	56	58-61	2002
天谷雅行	天疱瘡の分子生物学.	**現代医療**	34	1846-1851	2002

天谷雅行	天疱瘡と Dsg3 ノックアウトマウス.	Annual Review 2003 免疫		280-285	2002
天谷雅行	表皮細胞接着と皮膚疾患.	日本皮膚科学会雑誌	112	1569-1575	2002
松井稔, 舩田正彦	ムスカリン性受容体サブタイプと疾患 -最近の展開-.	精神保健研究	48	43-51	2002
長山成美、三宅幸子、山村 隆：	実験的自己免疫性脳脊髄炎（EAE）における NK 細胞/NK-LAK 細胞の体内動態および疾患抑制性の検討.	神経免疫学	10	104-105	2002
宮本勝一、三宅幸子、水野美歩、岡伸幸、山村 隆	実験的自己免疫性脳脊髄炎（EAE）の治療における選択的 COX-2 阻害剤の効果.	神経免疫学	10	106-107	2002
林 幼偉、三宅幸子、山村 隆	$CD4^+CD25^+$ T 細胞による実験的自己免疫性脳脊髄炎(EAE)の調節.	神経免疫学	10	108-109	2002
山村 隆	シンポジウム「日本における多発性硬化症の多様性とその病態」動物モデルからの提言.	臨床神経学	42	1201-1203	2002
山村 隆	多発性硬化症の 動物モデル.-実験的自己免疫性脳脊髄(EAE).	Current Insight s in Neurol Sci	10	10-11	2002
三宅幸子、山村 隆	NKT 細胞と実験的自己免疫性脳脊髄炎.免疫病研究の最先端. 分子制御というアプローチ.	Mebio	19	61-67	2002
荒木 学、高橋 和也、山村 隆	多発性硬化症における NK 細胞、NKT 細胞の関与.	神経内科	56:	312-318	2002
山村 隆	NKT 細胞と新しい自己免疫病治療薬.	Medical Science Digest	28	306-307	2002
宮本勝一、三宅幸子、山村 隆	糖脂質による自己免疫病の制御.	感染・炎症・免疫	32	200-201	2002
山村 隆、宮本勝一、長山成美、三宅幸子	NK・NKT 細胞による実験的自己免疫性脳脊髄炎（EAE）の発症制御.	蛋白質核酸酵素	47	1115-1120	2002
宮本 勝一、三宅 幸子、山村 隆	自己免疫性脳脊髄炎に対する NKT 細胞糖脂質リガンド療法.	神経免疫学	10	209-211	2002
天谷雅行	黄色ブドウ球菌毒素 ET の標的分子.	Medical Science Digest	29	254-255	2003
天谷雅行	自己免疫水疱症の最新情報.	日本医師会雑誌	129:	1425-1429	2003
天谷雅行	デスモグレインを標的とする疾患：天疱瘡とブドウ球菌性熱傷様皮膚症候群.	日本皮膚科学会雑誌	113	1947-1948	2003
天谷雅行	自己免疫性水疱症.	皮膚科の臨床	43	1343-1349	2003
松井 稔	ムスカリン性アセチルコリン受容体と脳神経機能 ノックアウトマウスを用いた成果	遺伝子医学	2003.6	47-50	2003
佐藤 準一、山村 隆	多発性硬化症におけるインターフェロンベータ療法の効果発現機序.	医療	57	441-455	2003
山村 隆	NKT 細胞と自己免疫. 調節性 $CD4^+$ NKT 細胞の役割.	Molecular Medicine	40	562-568	2003
山村 隆	多発性硬化症の発症機構と NK 細胞/NKT 細胞.	日本臨床	61	1329-1334	2003
山村 隆	NKT 細胞を介した自己免疫疾患制御.	炎症と免疫	11	616-622	2003
佐藤 準一, 山村 隆	多発性硬化症治療への新しい展望.	最新医学	58	1926-1938	2003

長山　成美, 山村　隆	抗コレステロール薬による Th2 優位の誘導 -MS の治療への応用は可能か？-.	臨床免疫	40	205-208	2003
長山　成美, 山村　隆	実験的自己免疫性脳脊髄炎と多発性硬化症. 特集 Molecular mimicry（分子模倣）と疾患.	医学のあゆみ	206	845-848	2003
山村　隆	近未来の多発性硬化症治療.	BIO Clinica	18	1069-1073	2003
山村　隆, 林　幼偉、三宅　幸子	多発性硬化症の進行を抑制する免疫細胞. 特集　脳と免疫.	Brain Medical	15	401-405	2003
天谷雅行	ELISA による天疱瘡自己抗体価測定法.	臨床皮膚科	58	84-88	2004
天谷雅行	水疱性疾患の自己抗体.	臨床検査	48	283-288	2004
天谷雅行	炎症性皮膚疾患の動物モデル：天疱瘡.	アレルギー科	17	136-140	2004
天谷雅行	ブドウ球菌性熱傷様皮膚症候群：SSSS.	小児科診療	67	391-395	2004
天谷雅行	天疱瘡.	アレルギー科	17	136-140	2004
角田和之, 天谷雅行	自己抗原ノックアウトマウスを用いた天疱瘡モデルマウス.	細胞工学	23	1198-1201	2004
天谷雅行	抗デスモグレイン自己免疫疾患・天疱瘡の病態解明-この 10 年の進歩-	細胞	36	477-480	2004
荒木　学、三宅幸子、山村　隆	多発性硬化症における NKT 細胞減少は長期ステロイド治療により補正される.	神経免疫学	12	175-179	2004
山村　隆	NKT 細胞のリガンドと Th1/Th2 バランス.	臨床免疫	41	14-17	2004
宮本　勝一, 山村　隆	多発性硬化症の新しい治療薬の開発.	Clinical Neuroscience	22:	847-850	2004
山村　隆	MS FRONTIER. 多発性硬化症の DNA マイクロアレイ解析.	Current Insights in Neurological Science	12	10-11	2004
大田孝幸, 天谷雅行	自己抗原ノックアウトマウスモデルからＢ細胞トランスジェニックマウスへ.	Molecular Medicine	印刷中		
佐藤　準一、山村　隆	多発性硬化症におけるインターフェロンベータ応答遺伝子.	Bio Medical Quick Review Net	印刷中		
山村　隆、高橋　和也、荒木　学	多発性硬化症と免疫調節細胞. 日本臨床　2005 年増刊.	臨床免疫学	印刷中		
山村　隆	多発性硬化症における免疫抑制薬の使い方：神経免疫疾患.	最新医学	印刷中		

Induction of Pemphigus Phenotype by a Mouse Monoclonal Antibody Against the Amino-Terminal Adhesive Interface of Desmoglein 3[1]

Kazuyuki Tsunoda,*† Takayuki Ota,* Miyo Aoki,* Taketo Yamada,‡ Tetsuo Nagai,† Taneaki Nakagawa,† Shigeo Koyasu,§ Takeji Nishikawa,* and Masayuki Amagai[2]*

Pemphigus vulgaris (PV) is a life-threatening autoimmune blistering disease that is caused by IgG autoantibodies against the cadherin-type adhesion molecule desmoglein (Dsg)3. Previously, we have generated an active mouse model for PV by adoptive transfer of Dsg3$^{-/-}$ splenocytes. In this study, we isolated eight AK series, anti-Dsg3 IgG mAbs from the PV mouse model, and examined their pathogenic activities in induction of blister formation. Intraperitoneal inoculation of the AK23 hybridoma, but not the other AK hybridomas, induced the virtually identical phenotype to that of PV model mice or Dsg3$^{-/-}$ mice with typical histology of PV. Epitope mapping with domain-swapped and point-mutated Dsg1/Dsg3 molecules revealed that AK23 recognized a calcium-dependent conformational epitope on Dsg3, which consisted of the V3, K7, P8, and D59 Dsg3-specific residues that formed the adhesive interface between juxtaposed Dsg, as predicted by the crystal structure. The epitopes of the mAbs that failed to show apparent pathogenic activity were mapped in the middle to carboxyl-terminal extracellular region of Dsg3, where no direct intermolecular interaction was predicted. These findings demonstrate the pathogenic heterogeneity among anti-Dsg3 IgG Abs due to their epitopes, and suggest the direct inhibition of adhesive interaction of Dsg as an initial molecular event of blister formation in pemphigus. ***The Journal of Immunology,*** **2003, 170: 2170–2178.**

Pemphigus vulgaris (PV)[3] is a life-threatening autoimmune disease that is characterized by suprabasal blisters on the skin and mucous membranes (1). Patients with PV develop IgG autoantibodies against desmoglein (Dsg)3, which is a desmosomal transmembrane glycoprotein that belongs to the cadherin superfamily of cell-cell adhesion molecules (2–4). Compelling evidence exists to indicate that IgG autoantibodies against Dsg3 play a pathogenic role in blister formation in PV. When whole IgG from patients' sera or IgG affinity purified on human recombinant Dsg (hDsg)3 were injected into neonatal mice, the mice developed intraepidermal blister formation with the typical histological features of PV (5–7). In addition, PV patients' sera, from which the anti-Dsg3 IgG was depleted by immunoadsorption with hDsg3, no longer induced blistering in neonatal mice (8).

The titers of serum anti-Dsg3 IgG autoantibodies, as measured by indirect immunofluorescence or ELISA, generally correlate with disease activity when monitored in individual patients (9–12). However, these titers are not absolute indicators for the severity of the disease among groups of patients, and it is sometimes the case that patients with low titers of anti-Dsg3 IgG autoantibodies show severe phenotypes, while patients with high titers show mild phenotypes (10, 11). It is not known whether all IgG autoantibodies that bind in vivo to the native Dsg3 are equally pathogenic, or whether each anti-Dsg3 IgG has a distinct potency for the induction of blister formation. Furthermore, if different autoantibodies have different pathogenic activities, what defines pathogenic strength at the molecular level? The major obstacle to addressing this question is that patients' sera contain polyclonal anti-Dsg3 IgG autoantibodies that react with different parts of the Dsg3 molecule (13, 14). Therefore, it is not feasible to compare the pathogenic activities of individual anti-Dsg3 IgGs using patients' sera. Although several reports describe the generation of human mAbs from patients (15–17), the isolation of a comprehensive set of human mAbs to compare differences in activities has proven difficult.

Recently, we developed an active disease mouse model for PV by using a novel approach, which involves autoantigen-knockout mice that do not acquire tolerance to the defective gene product (18). The adoptive transfer of splenocytes from mDsg3-immunized Dsg3$^{-/-}$ mice to Dsg3-expressing Rag2$^{-/-}$ recipient mice resulted in the stable production of anti-Dsg3 IgG and the development of PV phenotypes, which included oral erosions with typical PV histology. Thus, the PV model mice produced pathogenic anti-Dsg3 IgG Abs. To address the issue mentioned above, we used the PV model mice to generate a panel of anti-Dsg3 IgG mAbs that could bind in vivo to different parts of the native Dsg3. We then examined the pathogenic activities of these mAbs using a well-established passive transfer assay, as well as an ascites formation assay in adult mice, and we characterized the mAb-binding epitopes on Dsg3. In this study, we demonstrate that the in vivo binding of IgG to Dsg3 is not sufficient to cause blistering, and that

Departments of *Dermatology, †Dentistry and Oral Surgery, ‡Pathology, and §Microbiology and Immunology, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan

Received for publication October 8, 2002. Accepted for publication December 12, 2002.

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.

[1] This work was supported by Health Science Research Grants for Research on Specific Diseases from the Ministry of Health, Labor, and Welfare of Japan, and by Grants-in-Aid for Scientific Research and for the Development of Innovative Technology from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

[2] Address correspondence and reprint requests to Dr. Masayuki Amagai, Department of Dermatology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail address: amagai@sc.itc.keio.ac.jp

[3] Abbreviations used in this paper: PV, pemphigus vulgaris; AK, acantholytic keratinocyte; Dsg, desmoglein; ETA, exfoliative toxin A; hDsg, human recombinant Dsg; mDsg, mouse recombinant Dsg; PF, pemphigus foliaceus.

0022-1767/03/$02.00

Table I. *Primers used for PCR amplification of the domain-swapped hDsg molecules*[a]

Constructs	Primers for hDsg1		Primers for hDsg3	
	5′ primers	3′ primers	5′ primers	3′ primers
hDsg3$^{1-34}$/hDsg1$^{35-496}$	primer 1	primer 2	primer 3	primer 4
hDsg3$^{1-10}$/hDsg1$^{11-496}$	primer 5	primer 2	primer 3	primer 6

[a] Primer 1, 5′-ccaagcaacccagaaaatcacatacegcatctctggagta-3′; primer 2, 5′-ttactgc catccagttagctgaga-3′; primer 3, 5′-gacaaccatggggctcttccccagaactac-3′; primer 4, 5′-ttgccaaaccctgcagaatcaagttcgcagcagcctgtcgt-3′; primer 5, 5′-ctggtttgcagcacaatctgagt gaatcttggcaattgggttttcttt-3′; primer 6, 5′-tgctgcgaacttgattctgcagggtttggcaaatttcaccca-3′.

each anti-Dsg3 IgG has a distinct pathogenic potency. Furthermore, we show that the pathogenic mAb binds to the N-terminal adhesive interface of Dsg3, which is the functionally important region of the molecule, while other mAbs that lack apparent pathogenic activity bind to functionally less important regions of the molecule.

Materials and Methods

Mice

PV model mice were produced, as previously described (18). In brief, 6- to 10-wk-old Dsg3$^{-/-}$ mice were immunized s.c. with 5 μg of purified mouse recombinant Dsg (mDsg)3 in CFA, boosted twice for 2 wk i.p. with mDsg3 in IFA, and boosted twice for 2 wk with soluble mDsg3. Splenocytes from the immunized mice were adoptively transferred into C57BL/6 Rag2$^{-/-}$ mice (Central Institute for Experimental Animals, Tokyo, Japan) via the tail vein. Recipient mice with PV phenotype were fed with moistened food (Oriental Yeast, Tokyo, Japan) to improve food intake. Anti-Dsg3 Ab production was examined by ELISA and live keratinocyte staining. All mouse studies were approved by the animal ethics review board of Keio University.

Production of mAbs

Splenocytes were isolated from mice that had the active PV phenotype, and fused with P3 mouse myeloma cells (a kind gift from S. Tsukita (Department of Cell Biology, Kyoto University Faculty of Medicine)) at a ratio of 5:1 with PEG 4000 (Merck, Darmstadt, Germany), followed by selection with hypoxanthine aminopterin thymidine in the presence of 10% hybridoma cloning factor (IGEN, Gaithersburg, MD). Hybridoma cells were screened initially using mDsg3 ELISA, and positive clones were tested subsequently by live keratinocyte staining. Of 220 ELISA-positive clones, 11 clones were positive by live keratinocyte staining and 8 of them were further analyzed. Each clone was obtained by three replicates of the limiting dilution method. The isotypes of the mAbs were determined using the Isotyping Kit (Roche Diagnostics, Mannheim, Germany). The mAbs were purified from culture supernatants using the HiTrap rProtein A FF column (Amersham Bioscience, Piscataway, NJ).

Production of recombinant proteins

Some of the recombinant proteins used in this study have been described previously (10, 14). In this study, two domain-swapped and eight point-mutated hDsg1/hDsg3 molecules were produced using the baculovirus expression system. Four domain-swapped mDsg1/mDsg3 molecules were also produced (H. Anzai, Y. Fujii, T. Nishikawa, and M. Amagai, unpublished results). To generate the domain-swapped molecules, various regions of the hDsg1 and hDsg3 were PCR amplified with the appropriate primers (Table I), digested with either *NcoI*/*XhoI* for the hDsg1 fragments or *NcoI*/*Eco*RI for the hDsg3 fragments, and subcloned into the pQE-Tri-System vector (Qiagen, Hilden, Germany). To produce the point-mutated molecules, mutations were introduced by two-step PCR using the appropriate primers (Table II), as described previously (19). The PCR products were digested with either *NcoI*/*XhoI* for the Dsg1 fragments or *NcoI*/*Eco*RI for the Dsg3 fragments, and subcloned into the pQE vector. Recombinant proteins were obtained, as previously described (7, 20, 21). In brief, recombinant baculoviruses were obtained by cotransfection of each construct with Baculo Sapphire DNA (Orbigen, San Diego, CA) into cultured insect Sf9 cells. High five cells (Invitrogen, San Diego, CA) cultured in serum-free EX cell medium (JRH Bioscience, Lenexa, KS) were infected with the recombinant viruses, and incubated at 27°C for 3–4 days. Recombinant proteins produced in the culture supernatants were purified on TALON affinity metal resin (Clontech, Palo Alto, CA), according to the manufacturer's recommendation.

ELISA and live keratinocyte staining

The reactivities of acantholytic keratinocyte (AK) mAbs with mDsg3, mDsg1, hDsg3, and hDsg1 were measured by ELISA using the respective Dsg molecules, as described previously (10, 11, 18). Live keratinocyte staining was performed using the mouse keratinocyte cell line PAM212, as described previously (18).

Passive transfer assays using neonatal mice

To evaluate the pathogenic activities of the AK mAbs, we performed a passive transfer study with neonatal mice, as described previously (5, 7, 8). We injected AK mAbs (75–200 μg/mouse) alone or together with a small amount of total IgG that was prepared by ammonium sulfate precipitation of pemphigus foliaceus (PF) serum or a small amount of exfoliative toxin A (ETA), which specifically cleaves Dsg1 (22). The dosages of PF IgG and ETA were set at 50% of the minimum dose needed to induce gross blistering, i.e., 1 mg/mouse of PF IgG and 1 μg/mouse of ETA. Neonatal ICR mice at 12–24 h of age (body weight of 1.5–2.0 g) were used (Japan SLC, Shizuoka, Japan), and the skin was evaluated grossly and microscopically 18–24 h after injection. To evaluate microscopic blisters, the entire body skin was sectioned into six strips of ~3 mm in width. Blister formation was assessed as positive when two or more sites with suprabasilar acantholysis were noted in whole sections.

Ascites formation assay

We developed an assay using hybridoma cells to evaluate the pathogenic activities of AK mAbs in adult mice. We inoculated i.p. 5×10^6-1×10^7 hybridoma cells of each AK mAb into Rag2$^{-/-}$ mice that were primed with 2,6,10,14-tetramethyl-pentadecane (Wako Pure Chemical Industries, Osaka, Japan). The inoculated mice were monitored for ascites formation as well as the appearance of the PV phenotype, which was manifested by weight loss and patchy hair loss. Biopsies of the oral mucous membranes

Table II. *Primers used for PCR amplification of the point-mutated hDsg1/hDsg3 molecules*[a]

Constructs	Primers	Amino Acid Change
hDsg3-M1-2	7, 9, 11, 12	T 25 H, Y 28 C, Q 29 A, T 31 N, K 33 Q, I 34 V
hDsg3-M1-2-3	7, 9, 13, 14	T 25 H, Y 28 C, Q 29 A, T 31 N, K 33 Q, I 34 V, F 48 Y, V 53 I, D 54 N, K 55 Q, N 56 K
hDsg1-M1-2	8, 10, 15, 16	H 25 T, C 28 Y, A 29 Q, N 31 T, Q 33 K, V 34 I
hDsg1-M1-2-3	8, 10, 17, 18	Y 48 F, I 53 V, N 54 D, Q 55 K, K 56 N
hDsg3-M7	7, 9, 19, 20	V 3 I, K 7 A, P 8 A
hDsg3-M8	7, 9, 21, 22	D 59 E
hDsg1-M7	8, 9, 10, 23	I 3 V, A 7 K, A 8 P
hDsg1-M7-8	8, 10, 23, 24, 25, 26	I 3 V, A 7 K, A 8 P, E 59 D

[a] Primer 7, 5′-gacaaccatggggctcttccccagaactac-3′; primer 8, 5′-gcagccatggactg gagtttcttcagagta-3′; primer 9, 5′-cggactagtgaggaacatgggtgtgccagc-3′; primer 10, 5′-ttactgccatccagttagctgaga-3′; primer 11, 5′-ctggtttgcagcacaatctgagtgaatcttggcaatgggtttctt tt-3′; primer 12, 5′-gattgtgctgcaaaccagcaagttacctaccgaatctctggagtggga-3′; primer 13, 5′-atcaattcctactccagagatgcggtatgtaacttgctggtttgcagcacaatctgagtgaatcttggcaatgggtttctttttg ag-3′; primer 14, 5′-cgcatctctggagtaggaattgatcagccacacatatgggatcttgtcattaatcagaaaact ggagatattaacataacagcta-3′; primer 15, 5′-ctgggttgcttggtaatctgaagtaatttggcgattgggttc ctctt-3′; primer 16, 5′-gattaccaagcaacccagaaaatctaccgcatctctggagtaggaattg-3′; primer 17, 5′-atcgattcccactccagagattcggtaggtgatttttctgggttgcttggtaatctgaagtaatttggcgattgggttc ctctttga-3′; primer 18, 5′-cgaatctctggagtgggaatcgatcagccgccttttggaatctttgttgttgacaaa aacactggtgaaaattaatataacatccatag-3′; primer 19, 5′-cttctctgcaggcagcggcaaatttgatccattc acgttt-3′; primer 20, 5′-tgaatggatcaaatttgccgctgcctgcagagaaggagaa-3′; primer 21, 5′-aaaaacactggagaaattaacataacagc-3′; primer 22, 5′-tgttatgttaatttctccagtgttttgtc-3′; primer 23, 5′-tgaatgggtgaagttcgcaaaaccctgtcgtgaaggtgaa-3′; primer 24, 5′-cttcacga cagggttttgcgaaacttcaccattcacgttt-3′; primer 25, 5′-aaactggtgatattaatataacatcc-3′; primer 26, 5′-atattaatatcaccagttttctgatt-3′.

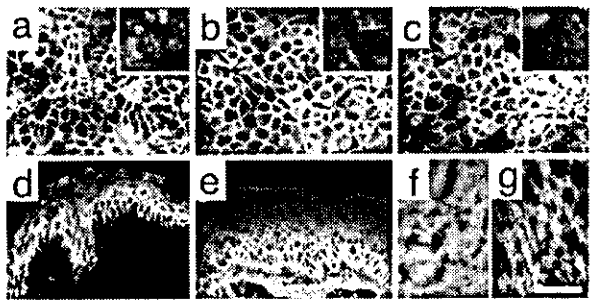

FIGURE 1. Immunoreactivities of the AK mAbs. The representative mAbs AK19 (*b*) and AK23 (*c*) bind to keratinocyte cell surfaces in similar patterns to that of polyclonal sera from PV model mice (*a*; *inset*, normal mouse serum), when added to cultures of mouse keratinocyte PAM212 cells. The reactivity of AK19 and AK23 mAbs was removed when those mAbs were immunoadsorbed with mDsg3-His (*insets* in *b* and *c*). AK23 mAb (*e*) as well as mouse sera from PV model mice (*d*) stain the lower parts of the human epidermis in which Dsg3 is expressed. AK23 did not stain the cell surfaces of epithelial cells in mouse heart (*f*) and liver (*g*) in which Dsg3 is not expressed. Bar = 50 μm.

and skin were taken when mice developed the PV phenotype or when ascites formation was observed after day 14.

Epitope mapping by immunoprecipitation

Immunoprecipitation was performed using the domain-swapped or point-mutated Dsg1/Dsg3 molecules to determine the mAb epitopes. Typically, 5 μg of purified mAb and 300 μl of culture supernatant that contained the recombinant proteins were mixed and incubated at room temperature for 30 min. Anti-E-tag mAb (Amersham Bioscience) was used as a positive control. The proteins were immunoprecipitated with protein G-Sepharose (Amersham Bioscience) at 4°C overnight. The immunoprecipitants were applied to SDS-PAGE and blotted onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The recombinant proteins were visualized with an anti-6× histidine Ab (R&D Systems, Minneapolis, MN). For the EDTA treatment, culture supernatants that contained the recombinant proteins were incubated with 5 mM EDTA for 30 min at room temperature, and subjected to immunoprecipitation, as described above.

Results

Generation of anti-Dsg3 IgG mAbs from PV model mice

PV model mice with active disease have circulating anti-Dsg3 IgGs that can induce the loss of cell-cell adhesion of keratinocytes with resultant blister formation. We used hybridoma cells from the splenocytes of PV model mice to produce anti-Dsg3 IgG mAbs. Initially, we screened the hybridomas by ELISA using mDsg3; positive clones were further screened by live staining of mouse keratinocyte PAM212 cells (Fig. 1, *a–c*). The second screening selected mAbs that could bind to the native Dsg3 on keratinocyte cell surfaces in vivo. Eight independent clones were isolated and designated as AK mAbs (Table III).

All of the mAbs had the IgG1 isotype for the H chain and the κ isotype for the L chain. Using indirect immunofluorescence with different tissue substrates, we found that all of these mAbs reacted with the cell surfaces of stratified squamous epithelia, such as those in the hard palate and skin, but not with simple epithelia, such as are found in the liver, heart, and intestine (Table III; Fig. 1, *f* and *g*). Some mAbs (AK1, 15, 18, 19, 20, and 23) cross-reacted with human skin or mucosa. These mAbs stained entire layers in the mucosa and the lower layers of the human epidermis in which Dsg3 was expressed (23, 24) (Fig. 1, *d* and *e*). All of the mAbs reacted exclusively, as assessed by ELISA, with the recombinant extracellular domain of the mDsg3, except AK1, which cross-reacted with mDsg1 (Table III). The mAbs that had detectable cross-reactivity by immunofluorescence with human tissues also reacted with hDsg3 in the ELISA (Table III).

Pathogenic activities of AK mAbs following passive transfer to neonatal mice

To determine whether the AK mAbs could induce blistering, we performed a passive transfer assay using neonatal mice, which is a well-established assay for pemphigus (5). Purified IgG from the culture supernatant of hybridoma cells was injected s.c. into neonatal mice, and the mice were observed for 18–24 h after injection (Table IV). The neonatal mice that were injected with AK19 or AK23, but not those that received the other mAbs, developed microscopic blisters with suprabasilar acantholysis in histology, although none of these mice developed apparent gross blisters (Fig. 2, *a* and *b*). The failure to induce gross blisters was not unexpected, because Dsg1 coexpression in the skin of neonatal mice compensates for the impaired adhesive function of Dsg3 (25, 26).

To overcome this problem, we used IgG preparations from PF sera that contained anti-Dsg1 IgG. We titrated the pathogenic strength of the PF IgG, and determined the minimum dose that induced gross blisters. Neonatal mice that were coinjected with AK19 and one-half of the minimum dose of PF IgG developed extensive gross blistering with suprabasilar acantholysis at 18–24 h (Fig. 2*e*), although mice that received PF IgG alone did not show blister formation (Fig. 2*c*). The neonatal mice that were coinjected

Table III. *Characterization of the AK mAbs*

		IIF[a]			ELISA								
		Mouse			Mouse		Human				Pathogenic Activity		
AK	Isotype	Mucosa	Liver	Human skin	Dsg3	Dsg1	Dsg3	Dsg1	Live Keratinocyte Staining	Ca Dependency[b]	Passive transfer	Ascites formation	Epitope[c]
1	IgG1 κ	+	−	+	+	+	+	+	+	−	−	−	ND
7	IgG1 κ	+	−	−	+	−	−	−	+	−	−	−	403–565
9	IgG1 κ	+	−	−	+	−	−	−	+	−	−	−	403–565
15	IgG1 κ	+	−	+	+	−	+	−	+	−	−	−	195–402
18	IgG1 κ	+	−	+	+	−	+	−	+	−	−	−	195–402
19	IgG1 κ	+	−	+	+	−	+	−	+	+	+	−	87–161
20	IgG1 κ	+	−	+	+	−	+	−	+	−	−	−	403–565
23	IgG1 κ	+	−	+	+	−	+	−	+	+	+	+	V3, K7, P8, D59

[a] Indirect immunofluorescence staining. Mouse oral mucous membrane (hard palate), mouse liver (the same negative staining was observed with heart and intestine), and human skin were used as the substrates.

[b] The reactivity of AK mAbs against mDsg3 was determined in the presence and absence of EDTA treatment. +, Indicates the abolition of activity by EDTA.

[c] The epitopes of AK7, AK9, AK15, AK18, and AK20 are indicated by the residue numbers for mDsg3. The AK19 epitope is indicated by the residue numbers for hDsg3. The AK23 epitope is conserved between the mouse and human Dsg3 proteins.

Table IV. *Summary of the pathogenic activities of AK mAbs*

	Passive Transfer[a]								Ascites Formation[b]			
	AK Alone				AK + PF IgG		AK + ETA					
			Blister formation			Gross		Gross		Titers of circulating anti-	In vivo IgG	PV
AK	*n*	IgG deposition	Microscopic	Gross	*n*	blisters	*n*	blisters	*n*	Dsg3 IgG[c]	deposition	phenotype
1	7	+	0	0	22	0	ND		10	ND	+	0
7	4	+	0	0	7	0	ND		10	ND	+	0
9	5	+	0	0	9	0	ND		10	ND	+	0
15	5	+	0	0	2	0	ND		2	289.1 ± 3.2	+	0
18	2	+	0	0	5	0	ND		5	21.1 ± 13.5	+	0
19	10	+	7	0	12	10	6	2	4	356.2 ± 10.4	+	0
20	6	+	0	0	10	0	ND		5	388.8 ± 9.6	+	0
23	5	+	5	0	7	7	6	6	9	64.5 ± 13.2	+	9

[a] AK mAb was s.c. injected alone or in combination with either PF IgG or ETA into neonatal mice, and the formation of gross or microscopic blisters was noted. All of the mice with gross blisters had the typical histologic features of PV.

[b] Hybridoma cells for each AK mAb were inoculated i.p. into $Rag2^{-/-}$ mice, and the development of the PV phenotype, which includes weight loss, patchy hair loss, and suprabasilar acantholysis in the oral mucosal membranes, was examined.

[c] Titers of circulating anti-Dsg3 IgG were measured by mDsg3 ELISA.

with AK23 and the same dose of PF IgG also had extensive blistering (Fig. 2*f*). No apparent blisters appeared following coinjection of mice with PF IgG and any of the remaining mAbs (data not shown).

We also took another approach to inactivate the adhesive function of Dsg1 in this neonatal mouse assay. Recently, it was shown that ETA, which is a serine protease that is produced by *Staphylococcus aureus*, specifically digests Dsg1 (22, 27). We titrated the activity of ETA in the manner described above. When either AK19 or AK23 was coinjected with one-half of the minimum effective dose of ETA, the mice demonstrated extensive blistering, although mice that were injected with ETA alone did not have any apparent blisters (Fig. 2, *d*, *g*, and *h*). Judging from the number of mice with gross blisters, AK23 appeared to be more potent than AK19 (Table IV). Blisters did not develop in mice that were coinjected with ETA and any of the remaining mAbs (data not shown).

These findings indicate that the AK19 and AK23 mAbs cause the loss of cell-cell adhesion of keratinocytes in neonatal mice, while the other AK mAbs do not have apparent blister-inducing activities.

Pathogenic activities of AK mAbs, as assessed by ascites formation in adult mice

We developed an assay using hybridoma cells to further determine whether AK mAbs showed pathogenic activity in inducing blisters in adult mice. We i.p. inoculated hybridoma cells into $Rag2^{-/-}$ immunodeficient mice, and evaluated the appearance of the PV phenotype, which was manifested by weight loss, patchy hair loss, and mucosal erosions (Table IV).

Mice that received AK23 hybridoma cells showed patchy hair loss and sudden mortality between days 7 and 9, which preceded ascites formation (Fig. 3, *a–c*). In these mice, in vivo IgG deposition was found on keratinocyte cell surfaces of stratified squamous epithelia, which included the oral and esophageal mucosal membranes and the skin, as was seen in both PV model mice and patients with PV (Fig. 3*e*). Histological examination of these mice revealed suprabasilar acantholysis in the hard palate (Fig. 3*g*) and in the skin around the snout. The development of oral erosions probably inhibited food and water intake, which led to dehydration and eventual death. Skin biopsies around the area of patchy hair loss showed intense IgG depositions on the cell surfaces of keratinocytes that surrounded the telogen hair club (data not shown), and cleft formation between the cells that surrounded the telogen club and the basal layer of the outer root sheath epithelium (Fig. 3*h*). The bald skin contained empty dilated telogen hair follicles. These gross, histologic, and immunopathologic findings were virtually identical with those observed in PV model mice, which indicates that AK23 is pathogenic and capable of regenerating the phenotype of the PV model mouse.

When hybridoma cells of the other AK mAbs (including AK19) were used, the murine PV phenotype did not develop, even after obvious ascites fluid formation at day 14 (Fig. 3*d*). Although all of the mice showed clear in vivo IgG deposition on keratinocyte cell surfaces in the stratified squamous epithelia, no blister formation was observed in the oral mucosa at the histological level (Fig. 3*f*; Table IV). The titers of circulating AK mAbs were measured by mDsg3 ELISA in mice that had apparent ascites formation (Table IV). For the mice that received AK23, the titers were measured when they showed signs of hair loss at days 7–9. The mAb titers

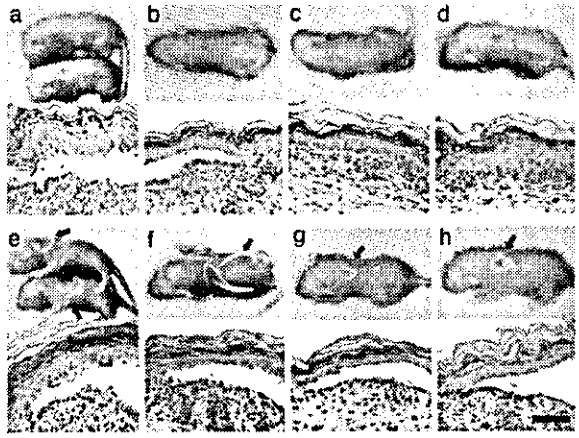

FIGURE 2. AK19 and AK23 induce PV blisters in the passive transfer assay. Neonatal mice that were injected with AK19 (*a*) or AK23 (*b*) developed microscopic blisters with suprabasilar acantholysis without apparent gross blistering. Neonatal mice that were coinjected with either AK19 (*e*) or AK23 (*f*) and PF IgG (*c*; in which the individual dosages were insufficient to induce blisters) developed extensive blistering (arrows) with suprabasilar acantholysis in histology. Neonatal mice that were coinjected with either AK19 (*g*) or AK23 (*h*) and ETA (*d*; in which the individual dosages were insufficient to induce blisters) developed extensive blistering (arrows) with the typical histology of PV. Bars = 50 μm.

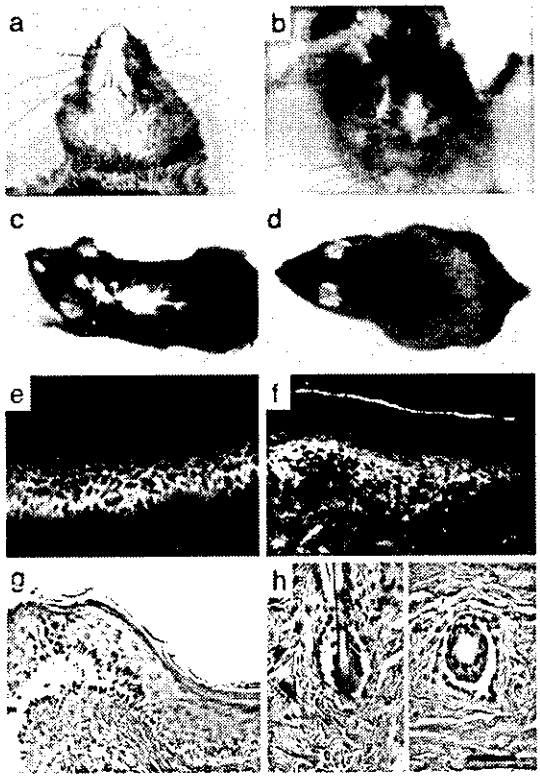

FIGURE 3. AK23 regenerates the phenotype of PV model mice. Recipient mice with AK23 hybridoma cells developed patchy hair loss around the mouth (*a*) and eyes (*b*), and on the back (*c*). This phenotype was not observed in mice that received the AK19 hybridoma cells, despite apparent ascites formation (*d*). Although in vivo IgG deposition on keratinocyte cell surfaces was observed in mice that received either AK23 (*e*, hard palate) or AK19 (*f*, hard palate) hybridoma cells, the loss of keratinocyte cell-cell adhesion was observed only in mice that received AK23 hybridoma cells (*g*, hard palate; *h*, skin, telogen hair club). Bars = 50 μm.

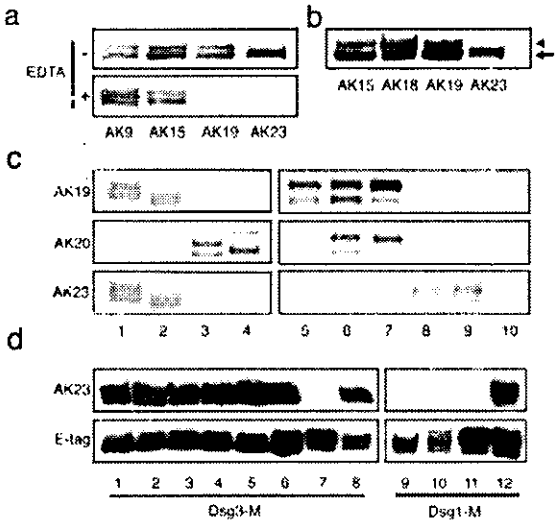

FIGURE 4. Immunoprecipitation of the domain-swapped and point-mutated Dsg1/Dsg3 recombinant proteins with AK mAbs. *a*, mDsg3-His was immunoprecipitated with AK mAbs with (+) or without (−) treatment with EDTA. AK19 and AK23 lost the ability to bind to Dsg3 after EDTA treatment. *b*, hDsg3-His was immunoprecipitated with the AK mAbs. AK23, but not the other AK mAbs, preferentially immunoprecipitated the lower band of the doublet. The arrow and arrowhead indicate the mature form of Dsg3 (after prosequence cleavage) and the immature form (with the prosequence), respectively. *c*, Immunoprecipitation of domain-swapped Dsg1/Dsg3 proteins with AK mAbs. *Lane 1*, $mDsg3^{1-162}$/$mDsg1^{163-512}$; *lane 2*, $mDsg3^{1-402}$/$mDsg1^{403-512}$; *lane 3*, $mDsg1^{1-194}$/$mDsg3^{195-565}$; *lane 4*, $mDsg1^{1-402}$/$mDsg3^{403-565}$; *lane 5*, $hDsg1^{1-24}$/$hDsg3^{25-566}$; *lane 6*, $hDsg1^{1-64}$/$hDsg3^{65-566}$; *lane 7*, $hDsg1^{1-87}$/$hDsg3^{87-566}$; *lane 8*, $hDsg3^{1-88}$/$hDsg1^{89-496}$; *lane 9*, $hDsg3^{1-63}$/ $hDsg1^{63-496}$; *lane 10*, $hDsg3^{1-34}$/$hDsg1^{35-496}$. *d*, Immunoprecipitation of point-mutated Dsg1/Dsg3 proteins with AK23 mAb (*upper panel*). Anti-E-tag mAb was used as a positive control (*lower panel*). *Lane 1*, hDsg3-M1; *lane 2*, hDsg3-M2; *lane 3*, hDsg3-M3; *lane 4*, hDsg3-M4; *lane 5*, hDsg3-M5; *lane 6*, hDsg3-M6; *lane 7*, hDsg3-M7; *lane 8*, hDsg3-M8; *lane 9*, hDsg1-M1–2; *lane 10*, hDsg1-M1–2-3; *lane 11*, hDsg1-M7; *lane 12*, hDsg1-M7–8.

of mice with AK15, 19, and 20 were at least 4-fold higher than those of mice with AK23, which excludes the possibility that the failure to induce blister formation was due to insufficient mAb production. These findings indicate that AK1, 7, 9, 15, 18, 19, and 20 are not sufficiently potent to induce the loss of cell-cell adhesion of keratinocytes in adult mice that have ascites formation.

Taken together, our findings indicate that AK23 induces blister formation with typical PV phenotype in both assays, whereas AK19 induces PV blisters in the passive transfer assay, but not in the ascites formation assay. The remaining mAbs lacked apparent pathogenic activities in either assay. Therefore, AK23 has the most potent pathogenic activity among the AK mAbs used in this study. AK19 also has pathogenic activity, but is weaker than AK23. The other AK mAbs have no apparent blister-inducing activities per se.

The pathogenic AK23 mAb recognizes a calcium-dependent conformational epitope consisted of V3, K7, P8, and D59 at the N terminus of Dsg3

To characterize the epitopes of the AK mAbs, we first determined whether mAb binding to Dsg3 was dependent on calcium. mDsg3-His was left untreated or treated with EDTA, and subjected to immunoprecipitation with the AK mAbs (Table III; Fig. 4*a*). EDTA treatment abolished the reactivities of the AK19 and AK23 mAbs, but not those of the other AK mAbs. When a mDsg3-coated ELISA plate was treated with EDTA, the binding of AK19 and AK23 was also abolished, while the binding of the other AK mAbs was not affected significantly (data not shown). Therefore, the AK19 and AK23 mAbs recognize calcium-dependent epitopes, while the other AK mAbs recognize calcium-independent epitopes.

Recombinant Dsg3-His is produced by baculoviruses as a doublet protein, in which the lower and upper bands are the mature (cleaved prosequence) and immature (uncleaved prosequence) forms, respectively (8, 10, 27). When the hDsg3-His was immunoprecipitated with AK mAbs as well as with anti-E-tag Abs, Dsg3 was detected as a doublet, except in the case of immunoprecipitation using AK23. Only the lower band was immunoprecipitated by AK23 (Fig. 4*b*). This finding suggests that AK23 mAbs, but not the other AK mAbs, bind preferentially to an epitope that only becomes available after cleavage of the prosequence.

We then attempted to map the epitopes of the AK mAbs by immunoprecipitation of domain-swapped Dsg1/Dsg3 recombinant molecules. It is believed that these domain-swapped Dsg1/Dsg3 molecules maintain the proper conformation, at least in terms of the binding of IgG autoantibodies, as determined previously (13, 14). Initially, we conducted gross mapping of the epitopes on Dsg3 using four domain-swapped molecules that contained the mDsg3 residues 1–162 ($mDsg3^{1-162}$/$mDsg1^{163-512}$), 1–402 ($mDsg3^{1-402}$/$mDsg1^{403-512}$), 195–565 ($mDsg1^{1-194}$/$mDsg3^{195-565}$), and

403–565 (mDsg1$^{1-402}$/mDsg3$^{403-565}$) (Figs. 4*c* and 5*a*). The AK1 epitope could not be determined using these domain-swapped molecules, because AK1 cross-reacted with both Dsg3 and Dsg1 (Table III). The AK7, AK9, and AK20 mAbs precipitated residues 195–565 and 403–565, but not residues 1–162 and 1–402. Therefore, the epitopes of AK7, AK9, and AK20 appear to reside in residues 403–565 of the mDsg3, which represents the C-terminal portion of the extracellular domain. The AK15 and AK18 mAbs reacted with residues 1–402 and 195–565, but not with residues 1–162 and 403–565. Therefore, the epitopes of AK15 and AK18 appear to be present in residues 195–402 of the mDsg3, which represents the middle portion of the extracellular domain. The AK19 and AK23 mAbs recognized residues 1–162 and 1–402, but not residues 195–565 and 403–565. Therefore, the epitopes of AK19 and AK23 appear to be present in residues 1–162 of the mDsg3, which represents the amino-terminal portion of the extracellular domain. The mAbs that cross-reacted with hDsg3 (AK15, 18, 19, 20, and 23) gave compatible mapping results with domain-swapped molecules using hDsg3 and hDsg1 (data not shown).

We identified more precisely the epitopes of AK19 and AK23, because these mAbs showed blister-induction activities. Because both of these mAbs cross-reacted with hDsg3, but not with hDsg1, and because their epitopes were at the amino terminus, we used eight additional domain-swapped hDsg3/hDsg1 molecules that contained the hDsg3 residues 25–566 (hDsg1$^{1-24}$/hDsg3$^{25-566}$), 65–566 (hDsg1$^{1-64}$/hDsg3$^{65-566}$), 87–566 (hDsg1$^{1-87}$/hDsg3$^{87-566}$), 1–88 (hDsg3$^{1-88}$/hDsg1$^{89-496}$), 1–63 (hDsg3$^{1-63}$/hDsg1$^{63-496}$), 1–34 (hDsg3$^{1-34}$/hDsg1$^{35-496}$), 1–26 (hDsg3$^{1-26}$/hDsg1$^{26-496}$), and 1–10 (hDsg3$^{1-10}$/hDsg1$^{11-496}$) (Fig. 5*b*). AK19 precipitated residues 87–566, but not residues 1–88 (Fig. 4*c*). Therefore, the AK19 epitope appears to be located in residues 87–161 of hDsg3. AK23 immunoprecipitated residues 1–88 and 1–63, which indicates that the AK23

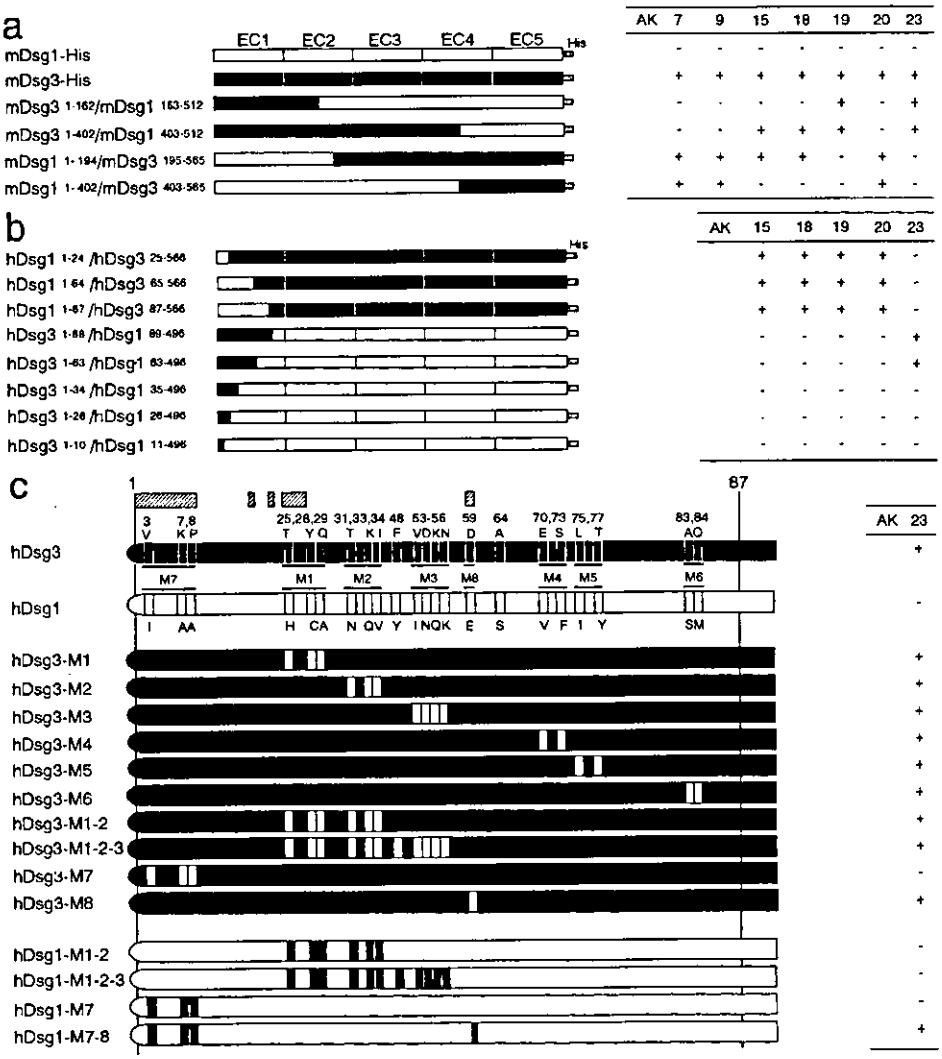

FIGURE 5. Molecular structures of the domain-swapped and point-mutated Dsg1/Dsg3 recombinant proteins, and their reactivities with the AK mAbs. Each of these constructs has a His-tag at the C terminus. The amino acid residues are numbered from the N terminus of the mature form of Dsg1 and Dsg3. The results of reactivity assays with AK mAbs are listed in the *right panel*. *a*, Domain-swapped mDsg1/mDsg3 molecules used in gross epitope mapping. *b*, Domain-swapped hDsg1/hDsg3 molecules used in fine epitope mapping. *c*, Point-mutated Dsg1/Dsg3 molecules used for epitope mapping of AK23. The 22 aa residues that are not conserved between hDsg1 and hDsg3 are indicated. Those nonconserved residues were switched between Dsg1 and Dsg3 to generate point-mutated molecules. The hatched boxes at the *top* indicate the residues that are predicted to form the adhesive interface of the W2 donor side (E1 to P8, P20, K23, T25, S26, D27, and D59) (31).

epitope resides in residues 1–63 of hDsg3 (Fig. 5*b*). However, because AK23 failed to react with residues 25–566, 1–34, 1–26, or 1–10, overlapping epitopes for AK23 could not be verified.

The amino-terminal sequences were highly conserved between Dsg3 and Dsg1. Only 22 aa residues of amino-terminal residues 1–87 were not conserved between the hDsg3 and hDsg1 proteins (Fig. 5*c*). We constructed a series of point-mutated hDsg3 molecules, in which the Dsg3-specific residues were replaced with the corresponding Dsg1-specific residues. We used six point-mutated Dsg3 (hDsg3-M1 to M6) constructs, which covered most of the Dsg3-specific residues between 25 and 84. However, none of these mutations led to loss of reactivity with AK23 (Figs. 4*d* and 5*c*). We then combined the M1 and M2 mutations to construct Dsg3-M1–2; however, AK23 still bound to Dsg3-M1–2. We replaced the Dsg3-specific residues 25–56 with the corresponding Dsg1-specific M1, M2, M3, and F48 residues to construct Dsg3-M1–2-3. However, AK23 still bound to Dsg3-M1–2-3. In contrast, AK23 did not bind to hDsg1-M1–2 or hDsg1-M1–2-3, which have the corresponding Dsg3-specific residues on the Dsg1 backbone. We then focused on the N-terminal residues, and point mutated the V3, K7, and P8 residues of Dsg3 to the I3, A7, and A8 residues of Dsg1 (hDsg3-M7). The hDsg3-M7 construct lacked reactivity with the AK23 mAb. However, the introduction of V3, K7, and P8 from Dsg3 onto the Dsg1 backbone (hDsg1-M7) was not sufficient to restore AK23 binding, which suggests that additional Dsg3-specific residues are necessary to form the AK23 epitope. When D59 was introduced into hDsg1-M7 (hDsg1-M7–8), hDsg1-M7–8 showed strong reactivity with AK23 (Fig. 4*d*). The substitution of D59 with E59 (hDsg3-M8) did not abolish reactivity with AK23. Thus, V3, K7, and P8 are essential residues for AK23 binding, and the combination of V3, K7, P8, and D59 is sufficient to generate the AK23 epitope on the Dsg1 backbone. The observed requirement for D59 in the formation of the AK23 epitope explains why the domain-swapped molecules that contained the residues 1–34, 1–36, and 1–10 of Dsg3 failed to react with AK23 (Figs. 4*c* and 5*b*). Thus, the epitope of AK23 maps to the V3, K7, P8, and D59 residues of Dsg3. All four of these Dsg3 residues are conserved between mice and humans.

Taken together, the epitope mapping of AK mAbs shows that the most potent mAb (AK23) recognizes an amino-terminal, calcium-dependent conformational epitope that is constituted by the V3, K7, P8, and D59 residues. The AK23 epitope becomes available only after cleavage of the prosequence. AK19, which shows weaker pathogenic activity, recognizes a calcium-dependent conformational epitope at residues 87–161 of Dsg3. The other AK mAbs, which lacked apparent pathogenic activities, recognize epitopes in the middle or carboxyl-terminal portions of the extracellular domain of Dsg3.

Discussion

It is well accepted that IgG autoantibodies to Dsg play a primary pathogenic role in blister formation on the skin and mucous membranes of pemphigus patients. The anti-Dsg3 IgG autoantibody titers correlate with disease activity when monitored in a single patient (9–12). However, when compared among different patients, the titers of anti-Dsg3 IgG autoantibodies do not necessarily reflect disease severity. In this study, we attempted to determine whether anti-Dsg3 IgG Abs had different pathogenic activities, and to define the factors that influenced pathogenic potency. To address this question, we isolated eight AK series mAbs against Dsg3 from active PV model mice (18). All of the AK mAbs were of the IgG1κ isotype and reacted in vivo with the cell surfaces of stratified squamous epithelia, but not with the surfaces of simple epithelia. AK7 and AK9 reacted with only mDsg3, while the others cross-reacted with hDsg3. The binding of AK19 and AK23 was calcium dependent, while that of the others was calcium independent.

We used two methods to evaluate the pathogenic activities of these mAbs. The first method involved passive transfer of mAbs into neonatal mice (5), and the second method assayed ascites formation in adult mice. The passive transfer assay may be more sensitive than the ascites formation assay because highly concentrated IgG can be applied in passive transfer, while the amount of IgG that can be used in the ascites formation assay is dependent on the production rate of each hybridoma. AK19 and AK23 induced blister formation after passive transfer, while the remaining mAbs failed to display pathogenic activities. In the ascites formation assay, only AK23 induced blisters, and the phenotypes of the mice that received AK23 hybridoma cells were virtually identical with those of PV model mice and Dsg3$^{-/-}$ mice. AK19 failed to induce blisters in the ascites formation assay, although the titers of circulating AK19 were more than 5-fold those of mice that were administered with the AK23 hybridoma cells. These findings indicate that AK23 and AK19 are capable of inducing both the loss of cell-cell adhesion of keratinocytes and blister formation with different potencies, while the other AK mAbs apparently lack pathogenic activities. This is the first demonstration of pathogenic heterogeneity among anti-Dsg3 IgG Abs that bind in vivo to the native Dsg3.

The epitopes of the AK mAbs were characterized by immunoprecipitation using domain-swapped as well as point-mutated Dsg1/Dsg3 molecules that were produced by baculovirus expression. Following immunoprecipitation with the domain-swapped molecules, the epitopes of AK7, AK9, AK15, AK18, and AK20, which lacked pathogenic activities, were mapped to the middle or carboxyl-terminal regions (between residues 195 and 565) of the extracellular domain of mDsg3. The epitopes of AK19 and AK23, which possessed pathogenic activities, were calcium dependent and located in residues 89–161 and 1–63, respectively. Subsequent extensive studies with point-mutated Dsg1/Dsg3 molecules revealed that AK23 recognizes a conformational epitope that consists of the V3, K7, P8, and D59 residues of Dsg3.

Cadherins are a family of calcium-dependent cell-cell adhesion molecules that play important roles in the formation and maintenance of complex tissues (28). Cadherins are classified into two major subgroups based on sequence similarities: classic cadherins (e.g., E-, P-, and N-cadherins) and desmosomal cadherins (Dsg and desmocollins). These cadherins share a common domain organization, which consists of five tandem extracellular cadherin domains (EC1 to EC5), a single transmembrane segment, and a highly conserved cytoplasmic domain (3, 4). Recent high resolution crystal structure analyses of classic cadherins have provided a mechanistic basis for intermolecular cadherin interactions (29, 30), and most recently, the crystal structure of the entire extracellular domain of C-cadherin, which is a member of the classic cadherin family, has been deduced with a resolution of 3.1 angstroms (31). This structure provides a new framework for understanding both the *cis* (same cell) and *trans* (juxtaposed cell) interactions of cadherin. The *trans* adhesive interface is a 2-fold symmetrical interaction that is defined by a conserved tryptophan (W2) side chain at the amino-terminal, membrane-distal end of the cadherin molecule from one cell, which inserts into the hydrophobic pocket at the amino-terminal end of a cadherin molecule on an opposing cell. This simple 2-fold symmetry provides a rationale for the generally observed homophilic specificity of cadherins, and reveals the molecular determinants of cadherin specificity.

When the amino acid sequences of Dsg3 were superimposed on the predicted structure of C-cadherin, the predicted residues for the adhesive interface of the W2 donor side were E1 to P8, P20, K23,

T25, S26, D27, and D59 (hatched boxes in Fig. 5*c*) (31). Among these residues, the ones that are not conserved between Dsg3 and Dsg1 and, therefore, probably involved in the determination of binding specificity of Dsg are V3, K7, P8, T25, and D59. Surprisingly, residues V3, K7, P8, and D59 of the epitope of AK23, which is the most potent pathogenic mAb tested, were located precisely on the Dsg3-specific residues of the adhesive interface. This structural prediction of the AK23 epitope is also supported by the observation that AK23 recognizes preferentially the mature form of Dsg3 (Fig. 4*b*). The prosequence of Dsg3 blocks the N-terminal adhesive region of cadherins to prevent self-aggregation during protein synthesis in the Golgi or endoplasmic reticulum (32). In contrast, the AK mAbs, which lacked apparent pathogenic activities, recognize the middle to C-terminal portion of the extracellular domain, a region in which no direct molecular interaction is predicted. Taken together, these findings indicate that the pathogenic heterogeneity among anti-Dsg3 IgG Abs in terms of blister formation is, at least in part, explained by their epitopes, and that the amino-terminal *trans* adhesive interface may represent a critical location for blister formation by IgG Abs in PV. Our findings also provide biological evidence for the predicted three-dimensional structure of cadherins (31).

Our findings do not necessarily exclude the possibility that Abs against the middle to carboxyl-terminal region of the extracellular domain of Dsg3 play some pathogenic role in blister formation. It is possible that the AK mAbs that lack apparent pathogenic activities per se may induce blisters when administered in combinations. When the epitopes of the anti-Dsg3 IgG autoantibodies were characterized using PV patients' sera, a minor proportion of the PV patients contained only IgG autoantibodies that were directed against the middle regions of Dsg3 (14). The clinical profiles of these patients were similar to those who had IgG autoantibodies against the N-terminal region of Dsg3. Therefore, polyclonal Abs against the middle portion of Dsg3 may also induce blistering.

The mechanism through which the binding of IgG Abs to Dsg3 leads to the loss of keratinocyte cell-cell adhesion is a matter of some controversy. In this respect, two hypotheses are currently favored: 1) direct inhibition of the adhesive function of Dsg3; and 2) the involvement of signal transduction. When IgG from PV sera was added to cultured keratinocytes, it caused a transient increase in intracellular calcium and inositol 1,4,5-triphosphate, which was followed by the activation of protein kinase C in the squamous cell carcinoma cell line DJM-1 (33). The addition of IgG from PV sera to cultured keratinocytes induced phosphorylation of Dsg3 and the dissociation of Dsg3 from plakoglobin (34). More convincingly, keratin retraction from cell-cell contact sites was induced by IgG from PV sera and required plakoglobin-mediated signaling in cultured mouse keratinocytes (35). These observations support the signal transduction hypothesis, although all of these observations were in vitro ones. In contrast, we demonstrated in this study that the most potent pathogenic mAb (AK23) recognized the functionally important N-terminal adhesive interface, while the other mAbs lacked apparent pathogenic activities bound to the functionally less important middle to C-terminal domain, showing that the pathogenic mAb does not simply cross-link the molecule or interfere with the desmosome through steric hindrance, but interferes with the actual function of Dsg3. In addition, the dominant epitopes in human PV were localized to the N-terminal residues 1–161 of Dsg3, which include the adhesive region (13, 14). The phenotype of mice with genetic disruption of Dsg3 is essentially identical with that of PV model mice (18, 36, 37). These findings indicate that the direct inhibition of the *trans* interaction of Dsg3 by IgG Abs is an initial molecular event of blister formation in pemphigus. Combining these two hypotheses, it is also possible to speculate that signal transduction may occur subsequent to the functional disruption of Dsg3 by IgG binding.

The importance of conformational epitopes was previously demonstrated in the production of pathogenic Abs by mouse immunization (38, 39) as well as epitope characterization with human PV sera (13, 14). The requirement of D59 residues in addition to V3, K7, and P8 residues to form the epitope of the pathogenic mouse mAb AK23 on Dsg1 backbone has ensured the importance of conformational epitopes in the pathogenesis of pemphigus. It was recently suggested that anti-Dsg Abs alone do not cause blistering, but require additional Abs to cholinergic receptors, and that pemphigus lesions can be caused by non-Dsg Abs (40–42). In addition to the extensive evidence that has been summarized previously (43), our findings that PV blisters could be induced by a single mAb (AK23) confirm that Dsg3 and anti-Dsg3 Abs are directly involved in causing blistering in PV, in the absence of any additional non-Dsg Abs.

The definition of the pathogenic hot spot on the molecule has provided an important framework to understanding the molecular mechanism of blister formation in pemphigus, as well as cell-cell adhesion in desmosomes. The availability of a series of mAbs against Dsg3 that serve as pathogenic and nonpathogenic Abs will provide a valuable tool to investigate the role of Dsg and desmosomes in keratinocyte differentiation and proliferation. Furthermore, from the diagnostic point of view, we should be able to develop an ELISA against the identified single epitope, which may provide absolute scores of disease severity. From the therapeutic point of view, the identification of pathogenic hot spot on Dsg3 will lead us to develop epitope-specific plasmapheresis or epitope-specific B cell elimination as targeted therapies for pemphigus.

Acknowledgments

We thank Dr. Shoichiro Tsukita for the mouse myeloma P3 cells and technical advice on the development of mAbs. Thanks also goes to Central Institute for Experimental Animals for $Dsg3^{-/-}$ mice and $Rag2^{-/-}$ mice. We also thank Yoshiko Fujii for the preparation of recombinant proteins, and Minae Suzuki for the immunofluorescence staining.

References

1. Stanley, J. R. 1993. Cell adhesion molecules as targets of autoantibodies in pemphigus and pemphigoid, bullous diseases due to defective epidermal cell adhesion. *Adv. Immunol. 53:291.*
2. Amagai, M., V. Klaus-Kovtun, and J. R. Stanley. 1991. Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. *Cell 67:869.*
3. Koch, P. J., and W. W. Franke. 1994. Desmosomal cadherins: another growing multigene family of adhesion molecules. *Curr. Opin. Cell Biol. 6:682.*
4. Amagai, M. 1995. Adhesion molecules. I. Keratinocyte-keratinocyte interactions, cadherins and pemphigus. *J. Invest. Dermatol. 104:146.*
5. Anhalt, G. J., R. S. Labib, J. J. Voorhees, T. F. Beals, and L. A. Diaz. 1982. Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. *N. Engl. J. Med. 306:1189.*
6. Amagai, M., S. Karpati, R. Prussick, V. Klaus-Kovtun, and J. R. Stanley. 1992. Autoantibodies against the amino-terminal cadherin-like binding domain of pemphigus vulgaris antigen are pathogenic. *J. Clin. Invest. 90:919.*
7. Amagai, M., T. Nishikawa, H. C. Nousari, G. J. Anhalt, and T. Hashimoto. 1998. Antibodies against desmoglein 3 (pemphigus vulgaris antigen) are present in sera from patients with paraneoplastic pemphigus and cause acantholysis in vivo in neonatal mice. *J. Clin. Invest. 102:775.*
8. Amagai, M., T. Hashimoto, N. Shimizu, and T. Nishikawa. 1994. Absorption of pathogenic autoantibodies by the extracellular domain of pemphigus vulgaris antigen (Dsg3) produced by baculovirus. *J. Clin. Invest. 94:59.*
9. Sams, W. M. J., and R. E. Jordon. 1971. Correlation of pemphigoid and pemphigus antibody titres with activity of disease. *Br. J. Dermatol. 84:7.*
10. Ishii, K., M. Amagai, R. P. Hall, T. Hashimoto, A. Takayanagi, S. Gamou, N. Shimizu, and T. Nishikawa. 1997. Characterization of autoantibodies in pemphigus using antigen-specific ELISAs with baculovirus expressed recombinant desmogleins. *J. Immunol. 159:2010.*
11. Amagai, M., A. Komai, T. Hashimoto, Y. Shirakata, K. Hashimoto, T. Yamada, Y. Kitajima, K. Ohya, H. Iwanami, and T. Nishikawa. 1999. Usefulness of enzyme-linked immunosorbent assay (ELISA) using recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. *Br. J. Dermatol. 140:351.*

12. Cheng, S. W., M. Kobayashi, A. Tanikawa, K. Kinoshita-Kuroda, M. Amagai, and T. Nishikawa. 2002. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmoglein 1 and 3. *Br. J. Dermatol. 147:261.*
13. Futei, Y., M. Amagai, M. Sekiguchi, K. Nishifuji, Y. Fujii, and T. Nishikawa. 2000. Conformational epitope mapping of desmoglein 3 using domain-swapped molecules in pemphigus vulgaris. *J. Invest. Dermatol. 115:829.*
14. Sekiguchi, M., Y. Futei, Y. Fujii, T. Iwasaki, T. Nishikawa, and M. Amagai. 2001. Dominant autoimmune epitopes recognized by pemphigus antibodies map to the N-terminal adhesive region of desmogleins. *J. Immunol. 167:5439.*
15. Sugi, T., T. Hashimoto, T. Hibi, and T. Nishikawa. 1989. Production of human monoclonal anti-basement membrane zone (BMZ) antibodies from a patient with bullous pemphigoid (BP) by Epstein-Barr virus transformation: analyses of the heterogeneity of anti-BMZ antibodies in BP sera using them. *J. Clin. Invest. 84:1050.*
16. Banchereau, J., P. de Paoli, A. Valle, E. Garcia, and F. Rousset. 1991. Long-term human B cell lines dependent on interleukin-4 and antibody to CD40. *Science 251:70.*
17. Peyron, E., J. F. Nicolas, A. Reano, P. Roche, J. Thivolet, M. Haftek, D. Schmitt, C. Peronne, J. Banchereau, and F. Rousset. 1994. Human monoclonal autoantibodies specific for the bullous pemphigoid antigen 1 (BPAg 1). *J. Immunol. 153:1333.*
18. Amagai, M., K. Tsunoda, H. Suzuki, K. Nishifuji, S. Koyasu, and T. Nishikawa. 2000. Use of autoantigen knockout mice to develop an active autoimmune disease model of pemphigus. *J. Clin. Invest. 105:625.*
19. Amagai, M., K. Ishii, A. Takayanagi, T. Nishikawa, and N. Shimizu. 1996. Transport to endoplasmic reticulum by signal peptide, but not proteolytic processing, is required for formation of conformational epitopes of pemphigus vulgaris antigen (Dsg3). *J. Invest. Dermatol. 107:539.*
20. Amagai, M., S. Karpati, V. Klaus-Kovtun, M. C. Udey, and J. R. Stanley. 1994. The extracellular domain of pemphigus vulgaris antigen (desmoglein 3) mediates weak homophilic adhesion. *J. Invest. Dermatol. 102:402.*
21. Amagai, M., T. Hashimoto, K. J. Green, N. Shimizu, and T. Nishikawa. 1995. Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. *J. Invest. Dermatol. 104:895.*
22. Amagai, M., N. Matsuyoshi, Z. H. Wang, C. Andl, and J. R. Stanley. 2000. Toxin in bullous impetigo and staphylococcal scalded skin syndrome targets desmoglein 1. *Nat. Med. 6:1275.*
23. Amagai, M., P. J. Koch, T. Nishikawa, and J. R. Stanley. 1996. Pemphigus vulgaris antigen (desmoglein 3) is localized in the lower epidermis, the site of blister formation in patients. *J. Invest. Dermatol. 106:351.*
24. Shirakata, Y., M. Amagai, Y. Hanakawa, T. Nishikawa, and K. Hashimoto. 1998. Lack of mucosal involvement in pemphigus foliaceus may be due to low expression of desmoglein1. *J. Invest. Dermatol. 110:76.*
25. Mahoney, M. G., Z. Wang, K. L. Rothenberger, P. J. Koch, M. Amagai, and J. R. Stanley. 1999. Explanation for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris. *J. Clin. Invest. 103:461.*
26. Amagai, M. 1999. Autoimmunity against desmosomal cadherins in pemphigus. *J. Dermatol. Sci. 20:92.*
27. Hanakawa, Y., N. M. Schechter, C. Lin, L. Garza, H. Li, T. Yamaguchi, Y. Fubada, K. Nishifuji, M. Sugai, M. Amagai, and J. R. Stanley. 2002. Molecular mechanisms of blister formation in bullous impegito and staphylococcal scalded skin syndrome. *J. Clin. Invest. 110:53.*
28. Takeichi, M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science 251:1451.*
29. Shapiro, L., A. M. Fannon, P. D. Kwong, A. Thompson, M. S. Lehmann, G. Grubel, J. F. Legrand, J. Als-Nielsen, D. R. Colman, and W. A. Hendrickson. 1995. Structural basis of cell-cell adhesion by cadherins. *Nature 374:327.*
30. Nagar, B., M. Overduin, M. Ikura, and J. M. Rini. 1996. Structural basis of calcium-induced E-cadherin rigidification and dimerization. *Nature 380:360.*
31. Boggon, T. J., J. Murray, S. Chappuis-Flament, E. Wong, B. M. Gumbiner, and L. Shapiro. 2002. C-cadherin ectodomain structure and implications for cell adhesion mechanisms. *Science 296:1308.*
32. Ozawa, M., and R. Kemler. 1990. Correct proteolytic cleavage is required for the cell adhesive function of uvomorulin. *J. Cell Biol. 111:1645.*
33. Kitajima, Y. 1996. Adhesion molecules in the pathophysiology of bullous diseases. *Eur. J. Dermatol. 6:399.*
34. Aoyama, Y., M. K. Owada, and Y. Kitajima. 1999. A pathogenic autoantibody, pemphigus vulgaris-IgG, induces phosphorylation of desmoglein 3, and its dissociation from plakoglobin in cultured keratinocytes. *Eur. J. Immunol. 29:2233.*
35. Caldelari, R., A. de Bruin, D. Baumann, M. M. Suter, C. Bierkamp, V. Balmer, and E. Muller. 2001. A central role for the armadillo protein plakoglobin in the autoimmune disease pemphigus vulgaris. *J. Cell Biol. 153:823.*
36. Koch, P. J., M. G. Mahoney, H. Ishikawa, L. Pulkkinen, J. Uitto, L. Shultz, G. F. Murphy, D. Whitaker-Menezes, and J. R. Stanley. 1997. Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. *J. Cell Biol. 137:1091.*
37. Ohyama, M., M. Amagai, K. Tsunoda, T. Ota, S. Koyasu, A. Umezawa, J. Hata, and T. Nishikawa. 2002. Immunologic and histopathologic characterization of active disease mouse model for pemphigus vulgaris. *J. Invest. Dermatol. 118: 199.*
38. Memar, O., B. Christensen, S. Rajaraman, R. Goldblum, S. K. Tyring, M. M. Brysk, D. J. McCormick, H. Zaim, J. L. Fan, and B. S. Prabhakar. 1996. Induction of blister-causing antibodies by a recombinant full-length, but not the extracellular, domain of the pemphigus vulgaris antigen (desmoglein 3). *J. Immunol. 157:3171.*
39. Fan, J. L., O. Memar, D. J. McCormick, and B. S. Prabhakar. 1999. BALB/c mice produce blister-causing antibodies upon immunization with a recombinant human desmoglein 3. *J. Immunol. 163:6228.*
40. Nguyen, V. T., T. X. Lee, A. Ndoye, L. D. Shultz, M. R. Pittelkow, M. V. Dahl, P. J. Lynch, and S. A. Grando. 1998. The pathophysiological significance of nondesmoglein targets of pemphigus autoimmunity: development of antibodies against keratinocyte cholinergic receptors in patients with pemphigus vulgaris and pemphigus foliaceus. *Arch. Dermatol. 134:971.*
41. Nguyen, V. T., A. Ndoye, and S. A. Grando. 2000. Pemphigus vulgaris antibody identifies pemphaxin: a novel keratinocyte annexin-like molecule binding acetylcholine. *J. Biol. Chem. 275:29466.*
42. Nguyen, V. T., A. Ndoye, L. D. Shultz, M. R. Pittelkow, and S. A. Grando. 2000. Antibodies against keratinocyte antigens other than desmogleins 1 and 3 can induce pemphigus vulgaris-like lesions. *J. Clin. Invest. 106:1467.*
43. Stanley, J. R., T. Nishikawa, L. A. Diaz, and M. Amagai. 2001. Pemphigus: is there another half of the story? *J. Invest. Dermatol. 116:489.*