

Human Mast Cell Transcriptome Project

Hirohisa Saito Toshioharu Nakajima Kenji Matsumoto

Department of Allergy and Immunology, National Children's Medical Research Center, Tokyo, Japan

Key Words

Genome · Mast cells · Oligonucleotide probe arrays · Transcriptome

Abstract

After draft reading of the human genome sequence, systemic analysis of the transcriptome (the whole transcripts present in a cell) is progressing especially in commonly available cell types. Until recently, human mast cells were not commonly available. We have succeeded to generate a substantial number of human mast cells from umbilical cord blood and from adult peripheral blood progenitors. Then, we have examined messenger RNA selectively transcribed in these mast cells using high-density oligonucleotide probe arrays. Many unexpected but important transcripts were selectively expressed in human mast cells. We discuss the results obtained from transcriptome screening by introducing our data regarding mast-cell-specific genes.

Copyright © 2001 S. Karger AG, Basel

Introduction

It came true at the beginning of 2001 that we are able to see all human genome sequences just by consulting the Internet [1, 2]. It was the year that we used to dream of in the last century, the departure of the starship 'Discovery' bound for the Jupiter. We will resolve previously unanswered questions such as the probability of diseases by utilizing the genome sequence. Now, we have a powerful

tool for establishing how genomic DNA sequence varies among individuals, and what the role of such a single nucleotide polymorphism (SNP) [3] is in the pathogenesis of diseases and responses to drugs. Comprehension of the genome will also accelerate the understanding of the transcriptome [4], the whole transcripts present in a cell, and the proteome [5] that controls the whole regulatory elements in a cell.

Until recently, it required much time and labor to measure expression levels of genes even for 100 transcripts. By the recent progress in technology, i.e. cDNA microarrays [6], high-density oligonucleotide probe arrays [7] and serial analysis of gene expression [8], for example, the systemic analysis for transcriptomes suddenly came true. Even in the past few years, such techniques are rapidly spreading all over the research fields [9, 10]. However, such a study on transcriptomes is mainly carried out with the cell types that can be easily obtained [11, 12] since transcriptome scanning still requires a large cell number.

Role of Human Mast Cells in Allergic Inflammation

Mast cells are the major effector cell type in the immediate-type allergic reaction [13], and are thought to evoke the late-phase inflammation through releasing cytokines acting on eosinophils [14, 15] and T cells [16]. Human mast cells are somewhat different from their rodent counterparts in terms of cytokine dependency [17, 18] and drug responses [19]. Video pictures of human mast cells activated through high-affinity IgE receptors (FcεRI) also

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
1018–2438/01/1251–0001\$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Hirohisa Saito, MD, PhD
Department of Allergy and Immunology, National Children's Medical Research Center
3-35-31 Taishido, Setagaya-ku, Tokyo 154-8509 (Japan)
Tel. +81 3 3414 8121, Fax +81 3 3414 8879
E-Mail hsaito@nch.go.jp

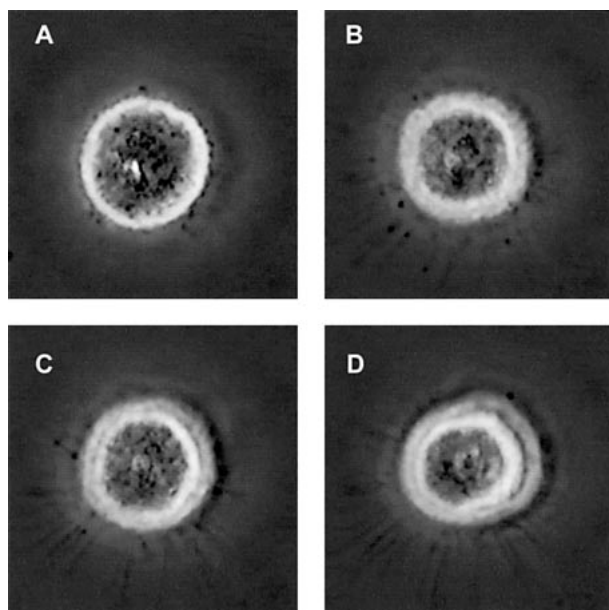


Fig. 1. Videotape images of human cultured mast cells activated via FcεRI. Cord-blood-derived human mast cells cultured for 14 weeks were challenged with anti-human IgE antibody after sensitization with human myeloma IgE under a videotape recorder. The images were captured 1 (**A**), 3 (**B**), 5 (**C**), and 10 (**D**) min after the challenge. The degranulation started approximately 1 min after stimulation. However, from 2 to 8 min, the mast cell often tried to retrieve granules by stretching long processes. The whole videotape image from 1 to 10 min after the challenge was clipped with mpeg-1 format to our URL [20].

seem to be quite different from the common image for degranulation that we may share. Some of the released granules are immediately captured and retrieved by long processes stretched from the cell (fig. 1) [20]. Indeed, when human mast cells suspended in liquid medium are challenged with anti-IgE, they release only limited amounts of protein mediators such as tryptase, while a substantial amount of low-molecular-weight histamine is released [21]. Thus, it is speculated that protein mediators may be working at very localized areas where mast cells are abundant. Since human mast cells have unique characters regarding cytokine dependency, responses to some drugs, and the pattern of degranulation, studies for medical application should be carried out using human counterparts.

Human mast cells can produce multiple cytokines through transcription of these genes for inducing allergic inflammation, i.e. tumor necrosis factor-α [22], granulocyte-macrophage colony-stimulating factor [23], macrophage inflammatory protein-1α [24], monocyte chemoattractant peptide-1 [25], fibroblast growth factor [26], and

interleukin (IL)-3 [27], IL-4 [28], IL-5 [29], IL-6 [27, 28], IL-8 [30], IL-9 [27], IL-10 [31], IL-13 [32, 33], and IL-16 [16] following IgE-dependent activation. Thus, a transcriptome assay for human mast cells seems to be of particular interest. However, so far, we could not easily obtain a substantial amount of human mast cells. Many procedures are necessary even to purify 10^5 cells from human tissue, while transcriptome assays such as cDNA microarrays require 10^8 cells. We have recently established the method of generating a substantial number of human mast cells from umbilical cord blood [21, 34] and from adult peripheral blood [35]. Among the expression screening arrays, high-density oligonucleotide probe arrays (GeneChip®, Affymetrix, Santa Clara, Calif., USA) require relatively small cell numbers ($<10^7$). The GeneChip® is designed to measure the absolute levels of $>10,000$ transcripts regardless of the cell type by using the same set of inner standards on a 1.2 cm^2 glass chip. The competition with another cell type required for cDNA microarray assay is not required with the GeneChip® [7, 11]. Thus, we were able to compare the expression levels of $>10,000$ transcripts even in different cell types such as cultured human mast cells derived from umbilical cord blood progenitors and from adult peripheral blood progenitors, and we discussed the different expression of high-affinity IgE [36]. In this series of studies, we selected the 89 genes specifically expressed in mast cells compared to peripheral blood leukocytes (table 1).

Human Mast-Cell-Specific Genes

We selected 89 transcripts present in human adult peripheral-blood-derived cultured mast cells by using high-density oligonucleotide probe arrays for 12,000 genes, and by screening those genes of $>5\%$ expression levels of housekeeping genes, >5 -fold increase compared to other leukocytes, and <5 -fold difference between two different samples. As expected, mRNA for tryptase, the most abundant protein, was ranked as the most increased transcript among human mast cell transcriptome (table 1). We could not determine the expression of the membrane type of tryptase [37], since it was found in 1999 and the present version of GeneChip (Human Genome U95A probe array) was designed based on the information for genes discovered until 1998. However, many other mediator genes expected to be mast-cell-specific, i.e. transcripts for *L*-histidine decarboxylase (18th increased transcript) responsible for histamine production, prostaglandin D2 synthase (5th increased transcript) and 15-

Table 1. Mast cell-specific transcripts (Top 1–89)

Rank	Accession No.	Name of transcript (protein)	Mast	Eo	MNC	Neu	Specificity	Subtracted
1	M33494	β -tryptase	210.0	0.9	0.8	1.2	218.2	207.1
2	M25915	clusterin	144.5	3.1	9.4	1.2	31.8	130.9
3	M16117	cathepsin G	88.3	0.7	0.7	<0.1	192.2	86.9
4	M63138	cathepsin D	109.7	9.2	20.5	12.9	7.8	67.3
5	AF150241	PGD ₂ synthase	67.1	<0.1	<0.1	<0.1	10,488.9	67.1
6	M73720	carboxypeptidase A	64.0	0.5	0.93	<0.1	137.6	62.6
7	AF002672	breast cancer suppressor candidate 1	54.8	<0.1	0.1	<0.1	1,392.2	54.6
8	X76534	glycoprotein (transmembrane) NMB	54.8	<0.1	0.13	<0.1	491.4	54.5
9	M68891	GATA-binding protein 2	66.1	4.8	8.5	0.9	14.0	51.9
10	U18009	membrane protein of cholinergic synaptic vesicles	52.5	2.6	6.7	1.3	15.0	41.9
11	AB006780	galectin-3	59.7	3.9	13.6	0.7	9.9	41.6
12	L76465	15 hydroxy-prostaglandin dehydrogenase	42.5	0.6	0.9	<0.1	87.8	41.1
13	D11139	tissue inhibitor of metalloproteinases	55.7	3.3	10.4	3.3	9.8	38.6
14	M89796	Fc ϵ R I β chain	38.0	0.2	0.5	<0.1	157.5	36.3
15	L78833	BRCA-1	42.3	2.2	3.3	2.3	16.2	34.4
16	R92331	metallothionein 1E	44.6	3.4	5.3	4.4	10.23	31.5
17	AF022813	NAG-2, transmembrane-4 superfamily	32.0	<0.1	5.5	<0.1	19.60	30.7
18	D16583	L-histidine decarboxylase	32.0	2.2	1.5	<0.1	26.29	28.3
19	AF001294	transmembrane-4 superfamily member 7	29.3	1.0	0.3	<0.1	62.65	27.9
20	M26683	interferon γ treatment inducible mRNA	32.9	4.6	0.5	1.0	16.1	26.8
21	X06182	c-kit proto-oncogene	26.4	<0.1	0.1	<0.1	472.4	26.2
22	D87119	GS3955/putative serine/threonine kinase	41.0	1.5	13.2	0.9	7.9	25.4
23	X56667	calretinin	28.5	1.5	0.8	1.3	24.0	24.9
24	M15518	tissue-type plasminogen activator	23.8	<0.1	<0.1	0.1	290.5	23.6
25	U78027	Bruton's tyrosine kinase	34.4	6.2	4.0	1.3	9.0	22.9
26	D86358	Siglec6	22.8	<0.1	0.2	<0.1	403.2	22.6
27	M69136	chymase	24.7	0.4	0.6	1.3	32.8	22.4
28	H68340	RNA helicase-related protein	27.3	1.7	3.5	<0.1	15.7	22.1
29	AL050224	RNA polymerase I	23.7	<0.1	1.1	1.2	30.7	21.4
30	L35594	dynactin subunit	21.2	0.2	0.1	<0.1	216.5	20.9
31	R93527	metallothionein 1H	33.9	3.8	5.6	3.6	7.8	20.9
32	M28225	MCP-1	23.0	2.1	0.3	0.6	23.3	20.1
33	U24576	breast tumor autoantigen (LMO4)	30.7	3.8	4.5	2.3	8.7	20.0
34	M94345	capping protein (actin filament), gelsolin-like	36.8	6.4	9.6	0.9	6.5	19.9
35	X51956	phosphopyruvate hydratase	21.5	0.2	2.0	<0.1	30.1	19.4
36	M93311	metallothionein 3	32.7	2.7	8.4	2.6	7.2	19.0
37	X75252	phosphatidylethanolamine binding protein	27.2	0.6	7.7	0.3	9.4	18.4
38	AF038844	MKP-1 like protein tyrosine phosphatase	17.5	<0.1	0.6	<0.1	85.4	16.9
39	U45448	P2x1 receptor	28.3	6.9	1.1	4.0	7.1	16.3
40	K01383	metallothionein 1A	19.8	<0.1	3.5	0.4	15.3	15.9
41	L07956	1,4- α -glucan branching enzyme	21.5	0.8	3.0	2.2	10.8	15.5
42	Z23090	28-kD heat shock protein	22.7	0.2	7.1	0.3	9.0	15.1
43	D13315	glyoxalase I	23.6	1.2	7.2	0.5	8.1	14.8
44	AB007937	KIAA0468 protein	22.7	1.6	3.0	4.1	7.8	14.0
45	X16396	NAD-dependent methylene tetrahydrofolate dehydrogenase cyclohydrolase (EC 1.5.1.15)	24.0	4.9	5.1	<0.1	7.2	14.0
46	M10943	metallothionein 1F	26.6	2.7	7.8	2.4	6.2	13.8
47	AL049957	cDNA DKFZp564J0323	17.3	0.4	1.4	1.8	14.3	13.7
48	AL035494	laminin receptor 1 (67kD)	22.3	3.8	5.6	<0.1	7.1	12.9
49	AB011123	KIAA0551 protein	15.6	1.4	1.0	0.6	15.4	12.6
50	X60435	PACAP	15.8	1.1	0.9	1.6	13.4	12.3
51	AB023137	A kinase (PRKA) anchor protein 2	15.3	1.3	1.8	<0.1	14.9	12.2
52	X62078	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	17.0	<0.1	3.9	1.1	10.1	12.0
53	AB025186	microtubule-associated protein, RP/EB family, member 3	18.2	1.0	1.5	3.8	8.7	11.9
54	AL048308	SIL, a gene frequently disrupted in T-cell acute lymphoblastic leukemia	12.1	<0.1	0.6	<0.1	50.8	11.4
55	W29087	56b8 homo sapiens cDNA	13.8	0.4	0.7	1.3	17.3	11.4
56	M13452	lamin A	12.0	0.7	0.3	<0.1	37.1	11.0
57	AJ011712	slow skeletal muscle troponin T	11.4	0.7	0.1	<0.1	40.6	10.6
58	X59798	human PRAD1 mRNA for cyclin D1	11.5	0.3	0.5	0.3	33.9	10.5
59	M24470	guanosine monophosphate reductase	14.1	0.8	1.1	2.6	9.4	9.6
60	Y11312	phosphoinositide 3-kinase	13.6	0.5	3.0	0.6	9.8	9.4
61	AF108145	MYLE mRNA, complete cds	14.6	0.5	4.4	0.3	8.4	9.4

Table 1 (continued)

Rank	Accession No.	Name of transcript (protein)	Mast	Eo	MNC	Neu	Specificity	Subtracted
62	X02344	ribosomal protein L24	21.8	3.9	4.3	4.3	5.3	9.3
63	U47634	human β -tubulin class III isotype	18.8	3.1	2.6	3.7	6.0	9.3
64	K03000	aldehyde dehydrogenase 1	13.7	0.2	4.4	<0.1	9.0	9.1
65	AA224832	metallothionein 1L	14.4	0.6	4.7	<0.1	8.1	9.1
66	X16665	homeo box B2	11.6	0.1	2.7	<0.1	12.5	8.8
67	U37022	human cyclin-dependent kinase 4 (CDK4)	11.2	<0.1	2.3	<0.1	14.1	8.8
68	D44466	mRNA for proteasome subunit p112	15.5	1.1	4.7	1.0	6.8	8.7
69	AF083105	HMG-box TF	9.8	0.4	0.6	0.3	22.5	8.5
70	AI547258	metallothionein 2A	13.5	1.7	2.4	1.1	7.7	8.3
71	M84349	CD59	10.3	1.0	1.0	1.0	10.4	7.3
72	AB002336	erythrocyte membrane protein band 4.1-like 1	9.1	0.4	0.5	1.2	13.6	7.1
73	U67369	growth factor independence-1 (Gfi-1)	8.2	0.2	1.0	<0.1	19.5	6.9
74	M24398	prathymosin	12.3	1.4	4.0	<0.1	6.9	6.9
75	U90907	human clone 23907 mRNA sequence	9.6	0.9	0.9	1.0	10.3	6.8
76	M73554	human bcl-1 mRNA	6.7	<0.1	<0.1	<0.1	1,653.8	6.7
77	U57093	small GTP-binding protein Rab27b mRNA	6.8	<0.1	0.2	<0.1	91.6	6.6
78	M82809	human annexin IV (ANX4) mRNA	10.9	0.3	3.5	0.6	7.5	6.5
79	X75593	RAB13, member RAS oncogene family	7.7	0.2	0.9	0.2	19.3	6.5
80	J04031	human methylenetetrahydrofolate dehydrogenase methenyltetrahydrofolate cyclohydrolase formyltetrahydrofolate synthetase mRNA	8.0	<0.1	1.4	0.4	13.2	6.2
81	L14269	vesicular monoamine transporter	6.2	0.2	0.1	<0.1	74.9	6.0
82	AL080181	immunoglobulin superfamily, member 4	6.0	<0.1	0.4	0.2	34.6	5.5
83	D50419	zinc finger protein 175	6.0	<0.1	0.6	<0.1	28.9	5.4
84	AL049969	cDNA DKFZp564A072	9.4	0.8	2.7	0.54	7.1	5.4
85	AL080061	chloride intracellular channel 4	9.1	0.8	2.9	<0.1	7.2	5.3
86	AB014526	KIAA0626 protein	6.3	<0.1	1.1	<0.1	18.6	5.3
87	U36601	heparan N-deacetylase/N-sulfotransferase-2	6.7	0.4	1.1	<0.1	14.1	5.3
88	U95740	chromosome 16 BAC clone CIT987SK-A-362G6	6.7	0.2	0.6	1.0	11.9	5.0
89	X12451	procathepsin L	9.6	2.4	2.1	0.3	6.2	5.0

The level of gene expression was obtained as average difference (AD) using GeneChip® software. Then, percentages of the specific AD level versus the mean AD level of 6 probe sets for housekeeping genes (β -actin and glyceraldehyde-3-phosphate dehydrogenase) were calculated. The mean percent AD level obtained from cultured mast cells (Mast), eosinophils (Eo), mononuclear cells (MNC), and neutrophils (Neu) were shown. 1. GenBank accession number (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide>) of the transcript was shown. 2. Mast cell specificity of the transcripts was expressed as

x-fold change obtained by dividing the mast cell value (Mast) by the mean of 3 other transcript levels (Eo, MNC and Neu). The transcripts of >5-fold change of the mean levels of other leukocytes and >3-fold change of each of 3 leukocyte levels were selected in the table. 3. Subtracted values were obtained by subtracting the other transcript levels (Eo, MNC and Neu) from the mast cell transcript level (Mast). Increased transcripts were ranked based on these subtracted values. The redundant transcripts were excluded if they repeatedly appeared.

hydroxy-prostaglandin dehydrogenase (12th increased transcript) responsible for producing prostaglandin D2 [38], cathepsin G (3rd increased transcript), carboxypeptidase A (6th increased transcript) and chymase (27th increased transcript) [39, 40] were expressed at high levels. Two distinct molecules producing heparin that controls the expression of several mast cell proteases through posttranslational mechanisms [41] were also selected. They were 1,4- α -glucan branching enzyme (41st increased transcript), and heparan N-deacetylase/N-sulfotransferase-2 (87th increased transcript). On the other hand, the α chain of Fc ϵ RI α and 5-O-lipoxygenase for producing leukotriene C4 were eliminated since mononuclear cells containing 2–3% basophils and eosinophils also expressed

the transcripts at significant levels. Several unexpected and interesting genes were also excluded because of experimental variance, although it might be due to a donor's SNP and might be very important.

Clusterin, an anti-inflammatory multifunctional protein, also called apolipoprotein J [42, 43], ranked as the 2nd most increased transcript, and the protein was recently found in mast cells. Clusterin present in mast cells is considered to play a role in the regression of hemangioma, the most frequent tumor of infancy [44]. Galectin-3 (11th increased transcript), an IgE binding glycoprotein, has already been found in protein form in a previous study [45].

Table 2. Specific transcripts preferentially expressed in cord blood-derived versus adult peripheral blood-derived cultured mast cells

Accession No.	Name of transcript (protein)	PB	CB	MNC	Specificity	Subtracted
<i>Increased transcripts in adults peripheral blood-derived versus cord blood-derived cultured mast cells and 3 other leukocytes</i>						
M12529	FcεR I β chain	29.4	6.2	0.5	4.8	22.7
D55696	chymase	22.1	6.7	0.6	3.3	14.8
X03084	FcεR I α chain	27.6	3.1	11.3	8.9	13.3
AF052124	CMAF leukocystatin	48.0	13.1	22.9	3.7	12.0
<i>Increased transcripts in cord blood-derived versus adult peripheral blood-derived cultured mast cells and 3 other leukocytes</i>						
M29877	α-L-fucosidase	7.1	31.6	5.2	4.4	19.3
M12529	apolipoprotein E	<16.3	<0.1	1,952.8	16.3	
D55696	cysteine protease	1.4	16.8	<0.1	12.1	15.3
X03084	C1q β-chain of complement system	<0.1	9.1	<0.1	1,086.2	9.1
AF052124	clone 23810, osteopontin	1.1	9.8	<0.1	7.53	7.3

The level of gene expression was obtained as average difference (AD) using GeneChip® software. Then, percentages of the specific AD level versus the mean AD level of 6 probe sets for housekeeping genes (β-actin and glyceraldehyde-3-phosphate dehydrogenase) were calculated. The mean percent AD level obtained from peripheral blood-derived cultured mast cells (PB) or cord blood-derived cultured mast cells (CB) in comparison to each other and mononuclear cells (MNC) were shown. 1. GenBank accession number (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide>) of the

transcripts was shown. 2. Specificity of the transcripts was expressed as 8-fold change obtained by dividing the mast cell value (PB or CB) by the mean of 2 other transcript levels (either one of CB or PB, plus MNC). The transcripts of >3-fold change of the mean levels of other cell types were selected in the table. 3. Subtracted values were obtained by subtracting the 2 other transcript levels from the mast cell transcript level (PB or CB). The levels of transcripts which varied (>5-fold) in experiments were also excluded. Only 9 genes satisfied the above criteria.

Breast cancer suppressor candidate 1 (7th increased transcript) [46], BRCA-1 (15th increased transcript) [47], and breast tumor autoantigen (33rd increased transcript) [48] were supposed to regulate the development of breast cancer, but their expression has not been determined in human mast cells. It would be interesting to elucidate whether mast cells enhance or inhibit the tumor growth by utilizing these proteins.

Of particular interest is that a variety of fibrinolytic and fibrinogenic enzymes responsible for tissue remodeling are expressed at high levels in mast cells. Tissue-type plasminogen activator (24th increased transcript), known to be useful for acute ischemic stroke [49], has been found in human mast cells [50]. Matrix metalloproteinases and their inhibitors (tissue inhibitor of metalloproteinases; 13th increased transcript) have been reported to play a role in tissue remodeling [51–53].

Six different isoforms of metallothioneins (16th, 32nd, 36th, 40th, 46th, and 70th increased transcripts), basically anti-apoptotic proteins [54], were selected in the mast-cell-specific genes. The expression of these proteins has been reported to be IL-6 dependent [55], suggesting that these proteins were just upregulated using a high concentration of IL-6 in mast cell culture medium [21].

A neuropeptide, calretinin (23rd increased transcript) is well documented in neurological journals [56, 57]. However, so far, they have not been studied in association with mast cells. Thus, the transcriptome assay may provide unexpected and potentially important results.

Specific Transcripts in Cord-Blood-Derived versus Peripheral-Blood-Derived Mast Cells

We have found that a single cord-blood-derived progenitor is capable of producing 15,000 mast cells, whereas a single peripheral progenitor produces only 800 mast cells. However, peripheral-blood-derived cultured mast cells are far more active than cord-blood-derived mast cells in terms of FcεRI-mediated reactions. Using high-density oligonucleotide probe arrays and serial analysis of gene expression, we found that α and β chains of FcεRI [36] and chymase levels [35] of cord-blood-derived cultured mast cells are selectively downregulated compared to adult peripheral-blood-derived counterparts even in the same culture environment. Therefore, IgE-mediated reactions may be restricted during early infancy via selective inhibition of FcεRI transcription. Yet, the two cell types were very similar, and only 6 other transcripts

were judged to be different in the two cell types beyond experimental variance, i.e. CMAP leukocystatin, α -L-fucosidase, apolipoprotein E, cysteine protease, C1q β -chain of complement system, and osteopontin (table 2).

CMAP leukocystatin, the increased transcript in peripheral-blood-derived mast cells compared to cord-blood-derived counterparts, is reported to play a role in immune regulation [58]. Five transcripts specifically increased in cord-blood-derived mast cells. Among them, α -L-fucosidase deficiency coincided with recurrent sinopulmonary infection and otitis media in addition to paronychia and a periapical dental abscess [59], suggesting that the significance of this protein in innate immunity. Since 1986, over one thousand studies have been published on osteopontin, the transcript increased in cord-blood-derived mast cells. These studies characterized osteopontin as a cell-adhesive, signaling, migratory, and survival stimulus for various mesenchymal, epithelial, and inflammatory cells, in addition to being a potent regulator of osseous and ectopic calcification. Therefore, osteopontin acts as a regulator of inflammation and biomineralization [60]. SNPs of the apolipoprotein E gene are known to affect several important diseases [61]. Recently, the protein has been found to protect against the lethal effects of bacterial lipopolysaccharides during sepsis caused by Gram-negative bacteria [62].

Conclusion

Thus, the critical information evidenced by transcriptome screening is often found incidentally in studies. Such accidental findings may have a profound effect on our understanding of cell biology as well as experimental procedures. Some investigators may publish a scientific original paper just by computing and comparing the transcriptome data published by others. Regarding mast cell biology, we did not yet demonstrate the data of transcriptomes expressed before and after Fc ϵ RI-dependent stimulation. However, according to our personal communication with a few mast cell biologists, such a human mast cell transcriptome study may be in progress. In the near future, we will know systematic features of transcripts and proteins present in activated and resting mast cells and whether or not findings in rodent mast cells are applicable to human mast cell biology.

Acknowledgment

We would like to thank the staff of the Department of Allergy and Immunology, National Children's Medical Research Center, for their excellent technical assistance on bioinformatics.

References

- 1 Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortmanet JR, et al: The sequence of the human genome. *Science* 2001;291:1304–1351.
- 2 International Human Genome Sequencing Consortium: Initial sequencing and analysis of the human genome. *Nature* 2001;409:860–921.
- 3 Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES: Characterization of single-nucleotide polymorphisms in coding regions of human genes. *Nat Genet* 1999;22:231–238.
- 4 Velculescu VE, Madden SL, Zhang L, Lash AE, Yu J, Rago C, Lal A, Wang CJ, Beaudry GA, Ciriello KM, Cook BP, Dufault MR, Ferguson AT, Gao Y, He TC, Hermeking H, Hiraldo SK, Hwang PM, Lopez MA, Luderer HF, Mathews B, Petroziello JM, Polyak K, Zawel L, Zhang W, Zhang X, Zhou W, Haluska FG, Jen J, Sukumar S, Landes GM, Riggins GJ, Vogelstein B, Kinzler KW: Analysis of human transcriptomes. *Nat Genet* 1999;23:387–383.
- 5 Apweiler R, Biswas M, Fleischmann W, Kanapin A, Karavidopoulou Y, Kersey P, Kriventseva EV, Mitterand V, Mulder N, Phan I, Zdobnov E: Proteome Analysis Database: Online application of InterPro and CluStr for the functional classification of proteins in whole genomes. *Nucleic Acids Res* 2001;29:44–48.
- 6 Duggan DJ, Bittner M, Chen Y, Meltzer P, Trent JM: Expression profiling using cDNA microarrays. *Nat Genet* 1999;21:10–14.
- 7 Lipshutz RJ, Fodor SPA, Gingeras TR, Lockhart DJ: High density synthetic oligonucleotide arrays. *Nat Genet* 1999;21:20–24.
- 8 Chen H, Centola M, Altschul SF, Metzger H: Characterization of gene expression in resting and activated mast cells. *J Exp Med* 1998;188:1657–1668.
- 9 Fambrough D, McClure K, Kazlauskas A, Lander ES: Diverse signaling pathways activated by growth factor receptors induce broadly overlapping, rather than independent, sets of genes. *Cell* 1991;97:727–741.
- 10 Alizadeh AA, Eisen MB, Davis RE, Ma C, Losos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JJ, Yang L, Marti GE, Moore T, Hudson J Jr, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO, Staudt LM: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;403:503–511.
- 11 Yoshioka K, Matsuda F, Takakura K, Noda Y, Imakawa K, Sakai S: Determination of genes involved in the process of implantation: Application of GeneChip to scan 6500 genes. *Biochem Biophys Res Commun* 2000;272:531–538.
- 12 Suzuki T, Hashimoto S, Toyoda N, Nagai S, Yamazaki N, Dong HY, Sakai J, Yamashita T, Nukiwa T, Matsushima K: Comprehensive gene expression profile of LPS-stimulated human monocytes by SAGE. *Blood* 2000;96:2584–2591.
- 13 Ishizaka T, Ishizaka K: Activation of mast cells for mediator release through IgE receptors; in Kallos P (ed): *Progress in Allergy*. Basel, Karger, 1984, vol 34, pp 188–235.

- 14 Tachimoto H, Ebisawa M, Kimata M, Mori K, Akasawa A, Nakajima T, Iikura Y, Saito H: Activated human mast cells release factors supporting the survival of eosinophils in vitro. *Int Arch Allergy Immunol* 1997;113:293–294.
- 15 Levi-Schaffer F, Temkin V, Malamud V, Feld S, Zilberman Y: Mast cells enhance eosinophil survival in vitro: Role of TNF- α and granulocyte-macrophage colony-stimulating factor. *J Immunol* 1998;160:5554–5562.
- 16 Mekori YA, Metcalfe DD: Mast cell-T cell interactions. *J Allergy Clin Immunol* 1999;104:517–523.
- 17 Saito H, Hatake K, Dvorak AM, Leiferman KM, Donnenberg AD, Arai N, Ishizaka K, Ishizaka T: Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. *Proc Natl Acad Sci USA* 1988;85:2288–2292.
- 18 Furitsu T, Saito H, Dvorak AM, Schwartz LB, Irani AMA, Burdick JF, Ishizaka K, Ishizaka T: Development of human mast cells in vitro. *Proc Natl Acad Sci USA* 1989;86:10039–10043.
- 19 Church MK, Hiroi J: Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol. *Br J Pharmacol* 1987;90:421–429.
- 20 English (mast cell project). <http://www.allergy.nch.go.jp/center>.
- 21 Saito H, Ebisawa M, Sakaguchi N, Onda T, Iikura Y, Yanagida M, Uzunaki H, Nakahata T: Characterization of human mast cells cultured in the presence of Steel factor and interleukin 6. *Int Arch Allergy Immunol* 1995;107:63–65.
- 22 Gordon JR, Galli SJ: Mast cells as a source of both preformed and immunologically inducible TNF- α /cachectin. *Nature* 1990;346:274–276.
- 23 Okayama Y, Kobayashi H, Ashman LK, Dobashi K, Nakazawa T, Holgate ST, Church MK, Mori M: Human lung mast cells are enriched in the capacity to produce granulocyte-macrophage colony-stimulating factor in response to IgE-dependent stimulation. *Eur J Immunol* 1998;28:708–715.
- 24 Tachimoto H, Ebisawa M, Hasegawa T, Kashiwabara T, Ra C, Bochner BS, Miura K, Saito H: Reciprocal regulation of cultured human mast cell cytokine production by IL-4 and IFN- γ . *J Allergy Clin Immunol* 2000;106:141–149.
- 25 Baghestanian M, Hofbauer R, Kiener HP, Bankl HC, Wimazal F, Willheim M, Scheiner O, Fureder W, Muller MR, Bevec D, Lechner K, Valent P: The c-kit ligand stem cell factor and anti-IgE promote expression of monocyte chemoattractant protein-1 in human lung mast cells. *Blood* 1997;90:4438–4449.
- 26 Qu Z, Liebler JM, Powers MR, Galey T, Ahmadi P, Huang XN, Ansel JC, Butterfield JH, Planck SR, Rosenbaum JT: Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *Am J Pathol* 1995;147:564–573.
- 27 Lorentz A, Schwengberg S, Sellge G, Manns MP, Bischoff SC: Human intestinal mast cells are capable of producing different cytokine profiles: Role of IgE receptor cross-linking and IL-4. *J Immunol* 2000;164:43–48.
- 28 Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, Howarth PH: Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993;151:3853–3865.
- 29 Jaffe JS, Glaum MC, Raible DG, Post TJ, Dimitry E, Govindarao D, Wang Y, Schulman ES: Human lung mast cell IL-5 gene and protein expression: Temporal analysis of upregulation following IgE-mediated activation. *Am J Respir Cell Mol Biol* 1995;13:665–675.
- 30 Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA: T helper cell type 2 cytokine-mediated mitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. *J Exp Med* 1999;190:267–280.
- 31 Ishizuka T, Okayama Y, Kobayashi H, Mori M: Interleukin-10 is localized to and released by human lung mast cells. *Clin Exp Allergy* 1999;29:1424–1432.
- 32 Kobayashi H, Okayama Y, Ishizuka T, Pawankar R, Ra C, Mori M: Production of IL-13 by human lung mast cells in response to Fc ϵ receptor cross-linkage. *Clin Exp Allergy* 1998;28:1219–1227.
- 33 Toru H, Pawankar R, Ra C, Yata J, Nakahata T: Human mast cells produce IL-13 by high-affinity IgE receptor cross-linking: Enhanced IL-13 production by IL-4-primed human mast cells. *J Allergy Clin Immunol* 1998;102:491–502.
- 34 Saito H, Ebisawa M, Tachimoto H, Shichijo M, Fukagawa K, Matsumoto K, Iikura Y, Awaji T, Tsujimoto G, Takahashi G, Yanagida M, Uzunaki H, Tsuji K, Nakahata T: Selective growth of human mast cells induced by Steel factor, interleukin 6 and prostaglandin E₂ from cord blood mononuclear cells. *J Immunol* 1996;157:343–350.
- 35 Ahn K, Takai S, Pawankar R, Kuramasu A, Ohtsu H, Kempuraj D, Tomita H, Matsumoto K, Akasawa A, Miyazaki M, Saito H: Regulation of chymase production in human mast cell progenitors. *J Allergy Clin Immunol* 2000;106:321–328.
- 36 Iida M, Matsumoto K, Tomita H, Nakajima T, Akasawa A, Ohtani NY, Yoshida NL, Matsui K, Nakada A, Sugita Y, Shimizu Y, Wakahara S, Nakao T, Fujii Y, Ra C, Saito H: Selective down-regulation of high affinity IgE receptor (Fc ϵ RI) α -chain messenger RNA among transcriptome in cord blood-derived versus adult peripheral blood-derived cultured human mast cells. *Blood* 2001;97:1016–1022.
- 37 Wong GW, Tang Y, Feyfant E, Sali A, Li L, Li Y, Huang C, Friend DS, Krilis SA, Stevens RL: Identification of a new member of the tryptase family of mouse and human mast cell proteases which possesses a novel COOH-terminal hydrophobic extension. *J Biol Chem* 1999;274:30784–30793.
- 38 Robinson C, Herbert CA, Bedwell S, Shell DJ, Holgate ST: The metabolism of prostaglandin D₂. Evidence for the sequential conversion by NADPH and NAD⁺ dependent pathways. *Biochem Pharmacol* 1989;38:3267–3271.
- 39 Schwartz LB: Mast cells: Function and contents. *Curr Opin Immunol* 1994;6:91–97.
- 40 Xia HZ, Kepley CL, Sakai K, Chelliah J, Irani AM, Schwartz LB: Quantitation of tryptase, chymase, Fc ϵ RI α , and Fc ϵ RI γ mRNAs in human mast cells and basophils by competitive reverse transcription-polymerase chain reaction. *J Immunol* 1995;154:5472–5480.
- 41 Humphries DE, Wong GW, Friend DS, Gurish MF, Qiu WT, Huang C, Sharpe AH, Stevens RL: Heparin is essential for the storage of specific granule proteases in mast cells. *Nature* 1999;400:769–772.
- 42 Calero M, Rostagno A, Matsubara E, Zlokovic B, Frangione B, Ghiso J: Apolipoprotein J (clusterin) and Alzheimer's disease. *Microsc Res Tech* 2000;50:305–315.
- 43 Newkirk MM, Apostolakis P, Neville C, Fortin PR: Systemic lupus erythematosus, a disease associated with low levels of clusterin/apoJ, an antiinflammatory protein. *J Rheumatol* 1999;26:597–603.
- 44 Hasan Q, Ruger BM, Tan ST, Gush J, Davis PF: Clusterin/apoJ expression during the development of hemangioma. *Hum Pathol* 2000;31:691–697.
- 45 Craig SS, Krishnaswamy P, Irani AM, Kepley CL, Liu FT, Schwartz LB: Immunoelectron microscopic localization of galectin-3, an IgE binding protein, in human mast cells and basophils. *Anat Rec* 1995;242:211–219.
- 46 Monaco C, Helmer Citterich M, Caprini E, Vorechovsky I, Russo G, Croce CM, Barbanti-Brodano G, Negrini M: Molecular cloning and characterization of ZNF202: A new gene at 11q23.3 encoding testis-specific zinc finger proteins. *Genomics* 1998;52:358–362.
- 47 Kent P, O'Donoghue JM, O'Hanlon DM, Kerin MJ, Maher DJ, Given HF: Linkage analysis and the susceptibility gene (BRCA-1) in familial breast cancer. *Eur J Surg Oncol* 1995;21:240–241.
- 48 Racevskis J, Dill A, Sparano JA, Ruan H: Molecular cloning of LMO41, a new human LIM domain gene. *Biochim Biophys Acta* 1999;1445:148–153.
- 49 Helmers SR: Issues in establishing primary stroke centers. *JAMA* 2000;284:1647.
- 50 Sillaber C, Baghestanian M, Bevec D, Willheim M, Agis H, Kapiotis S, Fureder W, Bankl HC, Kiener HP, Speiser W, Binder BR, Lechner K, Valent P: The mast cell as site of tissue-type plasminogen activator expression and fibrinolysis. *J Immunol* 1999;162:1032–1041.
- 51 Zhang J, Gruber BL, Marchese MJ, Zucker S, Schwartz LB, Kew RR: Mast cell tryptase does not alter matrix metalloproteinase expression in human dermal fibroblasts: Further evidence that proteolytically-active tryptase is a potent fibrogenic factor. *J Cell Physiol* 1999;181:312–318.

- 52 Di Girolamo N, Wakefield D: In vitro and in vivo expression of interstitial collagenase/MMP-1 by human mast cells. *Dev Immunol* 2000;7:131–142.
- 53 Tozzi CA, Thakker-Varia S, Yu SY, Bennett RF, Peng BW, Poiani GJ, Wilson FJ, Riley DJ: Mast cell collagenase correlates with regression of pulmonary vascular remodeling in the rat. *Am J Respir Cell Mol Biol* 1998;18:497–510.
- 54 Vasak M, Hasler DW: Metallothioneins: New functional and structural insights. *Curr Opin Chem Biol* 2000;4:177–183.
- 55 Penkowa M, Giralt M, Carrasco J, Hadberg H, Hidalgo J: Impaired inflammatory response and increased oxidative stress and neurodegeneration after brain injury in interleukin-6-deficient mice. *Glia* 2000;32:271–285.
- 56 Schulze E, Witt M, Fink T, Hofer A, Funk RH: Immunohistochemical detection of human skin nerve fibers. *Acta Histochem* 1997;99:301–309.
- 57 Diaz-Regueira S, Anadon R: Calretinin expression in specific neuronal systems in the brain of an advanced teleost, the grey mullet (*Chelon labrosus*). *J Comp Neurol* 2000;426:81–105.
- 58 Halfon S, Ford J, Foster J, Dowling L, Lucian L, Sterling M, Xu Y, Weiss M, Ikeda M, Liggett D, Helms A, Caux C, Lebecque S, Hannum C, Menon S, McClanahan T, Gorman D, Zurawski G: Leukocystatin, a new class II cystatin expressed selectively by hematopoietic cells. *J Biol Chem* 1998;273:16400–16408.
- 59 Ismail EA, Rudwan M, Shafik MH: Fucosidosis: Immunological studies and chronological neuroradiological changes. *Acta Paediatr* 1999;88:224–227.
- 60 Giachelli CM, Steitz S: Osteopontin: A versatile regulator of inflammation and biomineralization. *Matrix Biol* 2000;19:615–622.
- 61 Liestol K, Kvittingen EA, Rootwelt H, Dunlop O, Goplen AK, Pedersen JC, Brorson SH, Borresen-Dale AL, Myrvang B, Maehlen J: Association between apolipoprotein E genotypes and cancer risk in patients with acquired immunodeficiency syndrome. *Cancer Detect Prev* 2000;24:496–499.
- 62 Oosten Mv, Rensen PC, Amersfoort ES, Eck Mv, Dam AM, Breve JJ, Vogel T, Panet A, Berkel TJ, Kuiper J: Apolipoprotein E protects against bacterial lipopolysaccharide-induced lethality: A new therapeutic approach to treat Gram-negative sepsis. *J Biol Chem* 2001;276:8820–8824.

Copyright: S. Karger AG, Basel 2001. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.