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CASE REPORT

Altered Eosinophil Proteome in a Patient with Hypereosinophilia from Acute Fascioliasis▽

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We used comparative proteomics to analyze eosinophils from a patient with hypereosinophilia due to fascioliasis. Using 2-dimensional electrophoresis and mass spectrometry, we demonstrated that the eosinophil proteome was significantly altered compared to those of healthy controls.

CASE REPORT

A 48-year-old female presented to the Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, with complaints of colicky abdominal pain in the right upper quadrant. She had experienced symptoms for several years and had undergone a cholecystectomy 6 years before. She had previously worked in an area where fascioliasis is hyperendemic. One month prior to hospitalization, her pain increased in intensity, and symptomatic treatment did not alleviate the pain. On presentation, physical examination revealed a palpable liver 4 cm below the right costal margin and right upper quadrant abdominal tenderness. A complete blood count revealed an absolute eosinophil count of 3,060/μl. Subsequent blood counts confirmed hypereosinophilia. Bilirubin, alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were within normal limits. A computed tomography (CT) scan revealed small liver lesions. *Fasciola hepatica* infection (fascioliasis) was diagnosed by enzyme-linked immunosorbent assay (ELISA) (3), and the patient responded to triclabendazole with resolution of symptoms and improvement in eosinophilia over the next few weeks.

Prior to treatment, she consented to enroll in a study approved by the Institutional Review Board for research with human subjects at Universidad Peruana Cayetano Heredia. Granulocytes were isolated by gradient centrifugation and erythrocytes lysed with hypotonic saline as described previously (10). Granulocytes were incubated with anti-CD16 immunomagnetic beads and loaded onto a VarioMACS (Miltenyi Biotec, Auburn CA) column, and a magnetic field was used to separate neutrophils from eosinophils. The isolated eosinophils (>97% pure by Giemsa staining) were lysed in DeStreak rehydration buffer, frozen (−70 C), and transported on dry ice to the University of Texas Medical Branch, Galveston, TX, where the whole-cell lysate was resolved via 2-dimensional electrophoresis (2-DE) as described previously (10). Gels were stained with Sypro Ruby protein stain (Bio-Rad, Hercules CA) and imaged, and the normalized volumes of protein spots compared to a baseline eosinophil proteome map from control donors using Nonlinear SameSpots software (Nonlinear Dynamics, Durham NC). Proteins differentially expressed by ≥2-fold were excised and analyzed by matrix-assisted laser desorption ionization–tandem time of flight mass spectrometry (MALDI–TOF-TOF MS) (Applied Biosystems 4800) (10). Applied Biosystems GPS ExplorerTM (version 3.6) software was used along with MASCOT (Matrix Science, London, United Kingdom) to search protein databases using both MS and tandem MS (MS-MS) spectral data for protein identification.

Of over 3,000 protein spots identified by 2-DE, 60 were differentially expressed by ≥2-fold as measured by SameSpots analysis when compared to a healthy control profile averaged from 8 donors (Fig. 1) (10). Of the 60 protein spots that were differentially expressed, some 44 were subjected to MALDI–TOF-TOF MS, and their identifications are given in Table 1. Thirty-eight of the 44 had statistically significant expectation values of $<10^{-3}$ (10). Of the 38 significant proteins, 23 were unique within the data set after discounting redundant proteins. Redundant proteins typically represent a variety of posttranslational modifications that are frequently observed in eosinophils (10). They are usually represented by protein spots whose observed pIs and M_rs are among those that differ due to posttranslational modification.

Eosinophils are important mediators of allergies, asthma, and adverse drug reactions. In contrast, the adaptive role of eosinophils in human biology is thought to relate to host defenses against helminth infections (1, 6), which, even today, affect billions of people. Invasive infections with helminths are typically characterized by eosinophilia. However, eosinophil function in host defenses is incompletely understood.

In this preliminary analysis, we studied a single sample from a

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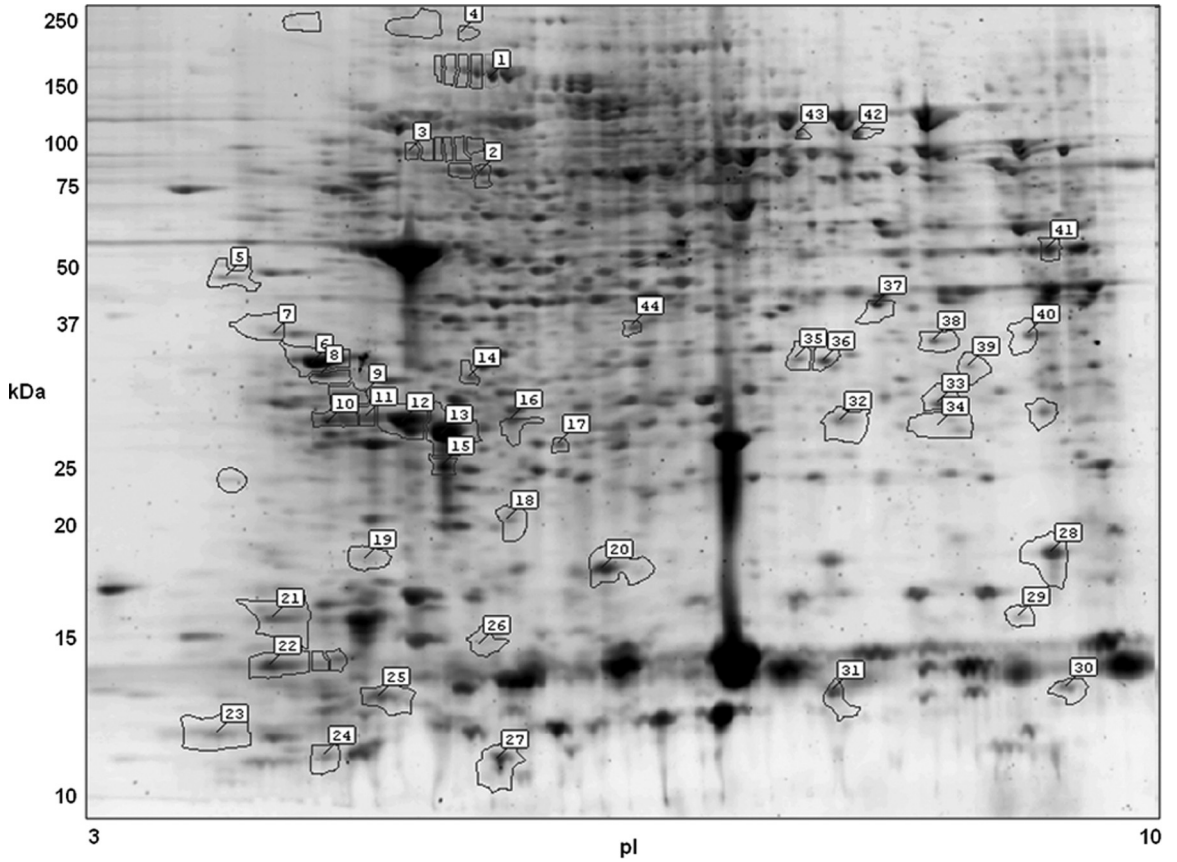

FIG. 1. Two-dimensional gel image of the eosinophils from a patient with *Fasciola hepatica* infection and hypereosinophilia, stained with Sypro Ruby fluorescent stain. Highlighted protein spots indicate proteins whose levels differed by at least 2-fold between the *Fasciola* patient and healthy controls. The spot numbers accompanying the highlighted proteins are the pick numbers in Table 1.

single time point and conducted comparative proteomic analysis of eosinophils using available data (~3,000 protein spots) from a healthy control (10). We noted that the level of expression of the vast majority of proteins was similar to that previously reported for quiescent peripheral blood eosinophils (control) (10). However, using a stringent statistical cutoff ($P < 0.001$), four proteins were statistically significantly upregulated in eosinophils from the *Fasciola* patient. These proteins included gelsolin, protein disulfide isomerase, neutral alpha-glucosidase, and the synaptic vesicle membrane protein *v*esicle *a*mine *t*ransport 1 (VAT-1). Protein disulfide isomerase has been shown to play a role in L-selectin (CD62L) shedding by activated eosinophils (2). Gelsolin is thought to play a role in degranulation (4) and adherence to target cells. The synaptic vesicle protein VAT-1 may play a role in antiapoptotic and prosurvival mechanisms, typical of several cytokines (11). The spot for ezrin was also increased, but this was not statistically significant. Ezrin is known to be expressed and phosphorylated in activated eosinophils (9). Among the downregulated proteins, annexin-1, S100-A9, and macrophage migration inhibitory factor are released during degranulation (7, 8). The downregulation of the observed protein spots could also have been due to mobility changes during 2-DE as a result of posttranslational modification, and thus, they may not represent downregulated proteins. We and others have observed that activated eosinophils show considerable phosphorylation (9, 13). Further studies using more patients are planned to evaluate phosphorylation of eosinophil proteins as a result of *Fasciola* infection. Proteomic studies of circulating eosinophils from patients with atopic dermatitis or pollen allergy did not correlate well with each other or the current study (12, 13). This suggests that the eosinophil proteome may differ depending on the route of sensitization or the allergen, even in patients with eosinophilia.

Using data from healthy controls, this proteomic analysis demonstrated that not only were eosinophil numbers increased but there was also a set of proteins that were differentially expressed during parasitic infection. These results suggest significant activation or priming of the eosinophil by the *Fasciola* infection. Similarly, Klion et al. report that compared to eosinophils from healthy donors, those isolated from individuals that suffer from parasitic infections appear to degranulate in the periphery and show upregulation of the cell surface markers CD69, CD25, and others (5).

This preliminary report points toward the usefulness of comparative proteomics in studying the effects of parasitic infections on eosinophils or other cells within the body. This approach revealed a set of novel proteins that were differentially expressed during *Fasciola* infection, which suggests the potential of this approach for proteomic investigations into eosinophil function. We propose that future unbiased characterization of a comprehensive set of proteins from different patients, modulated in the

TABLE 1. Proteins with altered expression in eosinophils from a patient with hypereosinophilia from fascioliasis

Protein name	SwissProt accession no.	Fold change[a]	Theoretical/observed		Peptide count	Expectation value[b]	Function	Pick no.[c]
			pI	M_r				
14-3-3 protein zeta/delta	P63104	2.1	4.73/5.17	27.9/21.31	12	1.58E-29	Cell signaling	10
60S ribosomal protein L4	P36578	5.6	11.07/7.6	47.95/73.91	12	0.16	Protein synthesis	42
Actin, cytoplasmic 2	P63261	4.9	5.31/4.91	42.11/11.06	4	1.26E-08	Actin-related protein	22
Actin, cytoplasmic 2	P63261	5.1	5.31/6.23	42.11/19.43	6	7.92E-16	Actin-related protein	17
Actin, cytoplasmic 2	P63261	2.8	5.31/5.7	42.11/20.2	9	3.97E-22	Actin-related protein	13
Actin, cytoplasmic 2	P63261	2.2	5.31/5.51	42.11/21.43	13	3.97E-42	Actin-related protein	12
Actin, cytoplasmic 2	P63261	2.5	5.31/5.35	42.11/21.89	12	1.26E-37	Actin-related protein	11
Actin, cytoplasmic 2	P63261	2.9	5.31/5.34	42.11/24.02	8	2.51E-29	Actin-related protein	9
Actin, cytoplasmic 2	P63261	4.0	5.31/5.82	42.11/24.93	10	6.29E-23	Actin-related protein	14
Actin, cytoplasmic 2	P63261	4.2	5.31/5.15	42.11/25	9	6.29E-28	Actin-related protein	8
Actin, cytoplasmic 2	P63261	2.0	5.31/5.1	42.11/25.09	12	1.58E-42	Actin-related protein	6
Actin-like protein 3	P61158	4.4	5.61/7.33	47.8/25.03	16	3.97E-25	Actin-related protein	35
Actin-like protein 3	P61158	3.1	5.61/7.46	47.8/25.07	15	5.00E-34	Actin-related protein	36
Alpha-enolase	P06733	2.7	7.01/6.58	47.48/24.16	15	9.98E-22	Carbohydrate metabolism	44
Annexin A1	P04083	3.2	6.57/7.98	38.92/23.24	14	9.98E-36	Ca^{2+}/phospholipid binding/exocytosis	33
Annexin A1	P04083	3.2	6.57/7.69	38.92/24.22	20	1.99E-43	Ca^{2+}/phospholipid binding/exocytosis	37
Annexin A1	P04083	6.1	6.57/8.12	38.92/25.1	6	7.92E-03	Ca^{2+}/phospholipid binding/exocytosis	39
Calreticulin precursor	P27797	2.4	4.29/4.7	48.28/27.01	5	1.99E-11	Ca^{2+}/phospholipid binding/exocytosis	5
Cofilin-1	P23528	2.1	8.22/6.44	18.72/15.89	12	6.29E-25	Actin-related protein	20
Cofilin-1	P23528	6.6	8.22/8.5	18.72/16.15	11	3.97E-20	Actin-related protein	28
Corticotropin-lipotropin precursor	P01189	2.3	7.56/5.34	29.8/16.05	4	6.29	Precursor for ACTH, MSH, beta-endorphin, and met-enkephalin	19
Ezrin	P15311	4.4*	5.94/5.87	69.48/12.49	6	0.16	Cytoskeletal protein	26
Fructose-bisphosphate aldolase A	P04075	5.2	8.3/8.49	39.85/34.54	14	1.58E-26	Carbohydrate metabolism	41
Gelsolin precursor	P06396	2.9*	5.9/5.92	86.04/85.18	26	3.97E-56	Actin-related protein	1
Guanine nucleotide-binding protein subunit beta 2-like 1	P63244	2.8	7.6/7.96	35.51/24.55	6	5.00E-06	Cell signaling	38
Heat shock protein HSP 90-alpha	P07900	2.2	4.94/6	85.01/16.56	7	1.58E-14	Molecular chaperone	18
Hemoglobin subunit beta	P68871	3.9	6.75/7.5	16.1/9.33	4	1.26E-07	Oxygen transport	31
Histone-lysine *N*-methyltransferase SETDB1	Q15047	5.0	5.74/5.7	145.12/18.12	15	0.20	Regulation of gene expression	15
Keratin, type I cytoskeletal 10	P13645	6.6	5.13/5.96	59.7/5	8	0.01	Intermediate filaments	27
Keratin, type I cytoskeletal 10	P13645	3.3	5.13/4.66	59.7/6.57	20	5.00E-28	Intermediate filaments	23
Keratin, type I cytoskeletal 9	P35527	4.3	5.19/8.35	62.32/14.29	10	5.00E-07	Intermediate filaments	29
Leukocyte elastase inhibitor	P30740	3.0	5.9/4.92	42.83/14.17	8	5.00E-26	Protease inhibition	21
Macrophage migration inhibitory factor	P14174	6.5	7.74/8.57	12.64/9.55	3	9.98E-05	Proinflammatory cytokine	30
Neuroepithelial cell-transforming gene 1 protein	Q7Z628	7.3	9.31/8.4	68.15/24.37	8	0.25	Cell signaling	40
Neutral alpha-glucosidase AB precursor	Q14697	2.2*	5.74/5.79	107.26/101.61	10	1.26E-08	Glycoprotein alteration	4
Plastin-2	P13796	2.4	5.2/4.93	70.82/24.33	9	1.99E-08	Actin-related protein	7
Protein disulfide-isomerase A3 precursor	P30101	2.1*	5.98/5.88	57.15/65.95	19	9.98E-42	Protein rearrangement/alteration	2
Protein disulfide-isomerase A3 precursor	P30101	2.0*	5.98/5.55	57.15/70.5	15	3.15E-14	Protein rearrangement/alteration	3
Protein S100-A9	P06702	2.4	5.71/5.16	13.29/5.22	4	6.29E-03	Ca^{2+} binding	24
Protein S100-A9	P06702	4.7	5.71/5.41	13.29/8.96	5	9.98E-06	Ca^{2+} binding	25
Synaptic vesicle membrane protein VAT-1 homolog	Q99536	3.9*	5.88/7.98	42.12/20.97	4	9.98E-03	Unknown function	34
Transketolase	P29401	5.2	7.58/7.34	68.52/73.91	17	9.98E-20	Carbohydrate alteration	43
Vimentin	P08670	4.7	5.06/7.53	53.68/21.54	23	1.99E-35	Mesenchymal intermediate filament	32
Vimentin	P08670	2.1	5.06/6.02	53.68/21.66	27	6.29E-29	Mesenchymal intermediate filament	16

[a] The protein in the *Fasciola* sample was upregulated in comparison to its level in the control; unmarked values were downregulated.
[b] Mascot-derived expectation score (9).
[c] Pick numbers are the spot numbers in Fig. 1.

course of parasitic infection, will provide novel insights into the molecular circuitry, signaling pathways, and cytokines that may play a role in the pathogenesis of parasite-induced eosinophilic inflammation.

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