Galectin-10, Eosinophils, and Celiac Disease

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Celiac disease (CD) is a chronic intestinal disease caused by intolerance to dietary wheat gluten in genetically susceptible individuals. There are a number of important open questions that impede the full explanation of the pathogenesis of this disease. We analyzed protein expression pattern in gut biopsies of CD subjects. Patients were selected and grouped according to histological inflammatory degree. Groups consisted of nine individuals with CD: three patients had a Marsh 0, three a Marsh I-II, and three a Marsh III. All CD patients showed a human leukocyte antigen DQ2/8 variant. Controls were three individuals with an excluded CD diagnosis. For the first time, galectin-10 expression was found related to the histological grade ($P = 0.0092$) and with the number of eosinophils in the lesion ($P = 0.0040$). Results suggest galectin-10 is a novel marker for evaluating CD tissue damage and eosinophils as a possible target for therapeutic approaches. Moreover, our data provide insights into alterations associated with CD tissue damage and pathogenesis.

Key words: **celiac disease; eosinophil; galectin-10; Charcot Leyden; 2D-DIGE**

Introduction

Celiac disease (CD) is a chronic intestinal disease caused by intolerance to dietary wheat gluten and related proteins in genetically susceptible individuals.[1,2] Although CD concerns approximately 1% of the population, most of the affected individuals remain undiagnosed. This is probably because patients with CD can manifest a spectrum of intestinal and/or extraintestinal symptoms.[3–5] Moreover, in some cases, CD patients can be relatively asymptomatic. The presumed disease is best detected by the serologic screening for the presence of IgA antibodies specific for tissue transglutaminase.[6–8] This should be followed by a biopsy of the mucosa of the small intestine to establish a definite diagnosis. The histological classification is based on the Oberhuber-modified Marsh classification: type I normal mucosa infiltrated with intraepithelial lymphocytes > 30/100 epithelial cells; type II with additional features of elongated or deeper crypts with increased mitoses consistent with crypt hyperplastic response; type III A-C with additional features of increasing atrophy of the villi from blunt to flat.[9] Life-threatening complications, although relatively rare, can include the development of refractory CD and enteropathy-associated T cell lymphomas (EATLs).[1]

Patients susceptible to CD present, in the large majority of cases, human leukocyte antigen (HLA)-DQ2/DQ8 variant molecules. Moreover, acquired T cell–mediated and innate responses play an important role in the pathogenesis of this disease.[2] Abnormal, monoclonal, intraepithelial lymphocytes are now

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Contemporary Challenges in Autoimmunity: Ann. N.Y. Acad. Sci. 1173: 357–364 (2009).
doi: 10.1111/j.1749-6632.2009.04627.x

associated with a subgroup of CD patients with refractory sprue, a severe complication of CD indicative for cryptic intestinal T cell lymphoma.[1] However, there are a number of important open questions and "missing links" that impede the full explanation of the pathogenesis of this disease. Advances in understanding the immunopathogenesis of CD could lead to the design of alternative treatment to gluten-free diet (GFD), which is currently the only accepted therapy for this disease and constitutes the background for identifying useful markers for diagnosis and identification of patients at risk for CD complications.

To investigate some of these important questions, it seems essential to develop proteomic approaches.

Materials and Methods

Patients

For proteomic analysis, duodenal biopsies were obtained from adult patients attending the Gastroenterology Unit of the Centro di Riferimento Oncologico (CRO), Istituto Di Ricovero e Cura a Carattere Scientifico (IRCCS), for suspected CD. Biopsies were fixed in Bouin's solution, and a portion of unfixed tissue was snap frozen in liquid nitrogen and stored at −80 °C for proteomic analysis. Histological evaluation was performed according to modified Oberhuber–Marsh classification.[9] HLA DQB1 PCR amplification and nucleotide sequences were carried out as previously reported.[10] Twelve individuals were selected for the study based on the clinical, molecular, and immunohistochemical results. Individuals showing at molecular analysis a γ or β oligoclonal/monoclonal T cell receptor chain rearrangement, indicative of an alteration in ongoing cellular immune response,[11] were excluded from the study. Three patients with excluded CD diagnosis constituted the case control group (patients' characteristics are reported in Table 1), the remaining nine subjects had clinically confirmed CD. Among these nine subjects, three had a Marsh 0, three a Marsh 1–2, and three a Marsh 3 histological classification at first visit. HLA DQ2/8 variants were present in all CD patients and absent in the three patients with excluded CD (Table 1).

TABLE 1. Characteristics of Patients

Patient	DQ2/8 variant	Modified Marsh grade	Celiac disease
Patient 1	DQ2	III A	Confirmed
Patient 2	DQ2	III A	Confirmed
Patient 3	DQ2	III A	Confirmed
Patient 4	DQ2	II	Confirmed
Patient 5	DQ2	I	Confirmed
Patient 6	DQ2	I	Confirmed
Patient 7	DQ2	0	Confirmed
Patient 8	DQ2	0	Confirmed
Patient 9	DQ2	0	Confirmed
Patient 10	–	0	Excluded
Patient 11	–	0	Excluded
Patient 12	–	0	Excluded

Patients were told the purpose of the study, and an informed consent was obtained.

Sample Preparation and Two-dimensional Differential In-gel Electrophoresis

To better understand pathogenetic mechanisms associated with CD and to search for a CD proteomic diagnostic signature, we analyzed differential protein expression patterns from gut epithelium by two-dimensional differential in-gel electrophoresis (2D-DIGE) analysis. The 2D-DIGE analysis was carried out as previously reported.[11] Briefly, proteins were extracted from gut biopsies with a sample grinding kit (GE Healthcare, Milan, Italy) and 200 μL of lysis buffer. The cell lysates were prepared for 2D-DIGE using a two-dimensional clean-up kit (GE Healthcare) and resuspended in 7 mol/L urea, 2 mol/L thiourea, and 4% CHAPS. Protein concentration was determined with the Bio-Rad protein assay (Bio-Rad Laboratories, Milan, Italy). For DIGE minimal labelling, 25 μg of protein sample was

mixed with 100 pmol CyDye (GE Healthcare) and incubated on ice in the dark for 30 min. Proteins were separated by two-dimensional electrophoresis by utilizing first-dimension reparation of isoelectric focusing (IEF; Bio-Rad Laboratories, Milan, Italy) cell using pH 3.0–10.00 nonlinear immobilized pH gradient strips (Bio-Rad Laboratories, Milan, Italy) Cell. For the second dimension, IPG strips were equilibrated in 7 mol/L urea, 2 mol/L thiourea, 2% SDS, 30% glycerol, 50 mmol/L Tris-HCl pH 8.8, reduced with 65 mmol/L dithiothreitol (DTT) and alkylated with 135 mmol/L iodoacetamide. The second dimension was run on Criterion IPG + 1 Comb 8–16% precast gels (Bio-Rad). Gels were scanned on a Typhoon TRIO scanner (GE Healthcare) at 100 μm resolution. Images were subjected to difference in-gel analysis (DIA) using DeCyder software version 6.5 (GE Healthcare), which normalized and statistically analyzed spots to identify and quantify differentially expressed proteins. Biological variation analysis (BVA) allowed matching of spots from multiple gels, calculated average abundance changes, and statistically analyzed the differential protein expression. Galectin-10 expression values were exported to an Excel worksheet with the XML toolbox tool (DeCyder software, GE Healthcare).

Protein Identification by MALDI-TOF Peptide Mass Fingerprinting

Protein spots of interest were excised from the colloidal Coomassie-stained preparative gel and destained with 25 mmol/L ammonium bicarbonate in 50% acetonitrile.[12] After overnight trypsin digestion, peptides were extracted with 1% trifluoroacetic acid (TFA) and then subjected to Zip Tip cleanup (Millipore, Milan, Italy). Peptide mass fingerprinting was performed on a Voyager-DE PRO Biospectrometry Workstation mass spectrometer (Applied Biosystems, Milan, Italy). Matrix-assisted laser desorption/ionization (MALDI) mass spectra were acquired in the 700–4000 Da molecular weight range, in positive ion mode, with 150-nsec delay time and an ion acceleration voltage of 20 kV. Spectra were externally calibrated using peptide calibration mix 4 (Proteomix) 500–3500 Da range (Laser Bio Labs, Sophia-Antipolis, France). Mass spectra, obtained by collecting 1000–2000 laser shots, were processed using Data Explorer version 5.1 software (Applied Biosystems). Peak lists have been obtained from the raw data following advanced baseline correction (peak width 32, flexibility 0.5, degree 0.1), noise filtering (noise filter correlation factor 0.7), and monoisotopic peak selection. Database searching was done with the online MASCOT search engine (http://www.matrixscience.com), Aldente (http://www.expasy.org/tools/aldente), and ProFound (http://prowl. rockefeller. edu/prowl-cgi/profound.exe) peptide mass fingerprinting tools against the National Center for Biotechnology Information (NCBI)nr and Swiss-Prot databases, limiting the search to human proteins, allowing for one trypsin missed cleavage, and with a 150 ppm mass tolerance error.

Molecular Analysis and Histology

HLA-DQB1 PCR amplification and nucleotide sequences were carried out as reported previously.[10]

Clonal T cell receptor γ- and β-chain variable regions were assessed previously by fluorescence multiplex PCR developed within the Biomed-2 concerted action and then subjected to capillary electrophoresis on an ABI prism 3100. Data were analyzed using 3100 GeneScan 3·7 software.[11,13]

All histology specimens at the CRO Institute were fixed by using both 10% formalin and Bouin's solution. From paraffin blocks, 5 μm-thick sections were cut and stained with conventional hematoxylin and eosin (H&E). Histological evaluation was performed according to the Marsh–Oberhuber classification.[9]

For each specimen, the high-power field (HPF) [Leica DMLB microscope ×10 ocular

(22 mm) and ×40 objective lens – PL FLUOTAR 40×/0.70: 0.237 mm^2; Leica, Wetzlar, Germany] was used to assess the maximum number of eosinophils per field; these were recorded as eos/HPF. Multiple sections of each specimen were evaluated, each section being totally examined.

Statistical Analysis

Multivariate one-way ANOVA analysis within DeCyder 2-D Differential Analysis Software was used for intergel matching and subsequent statistical analysis between all groups. GraphPad's InStat Statistical Software was used to perform the linear regression test. A P value less than 0.05 was used to determine statistical significance.

Results

We used the 2D-DIGE approach to search for a proteomic CD signature. After intragel analysis with the DIA module, we proceeded with intergel analysis with the BVA module of DeCyder software. Histopathologically, the protean manifestations of celiac disease display a range in severity. Depending on the gluten intake and stage of evolution of the disease, varying degrees of histological abnormalities may be seen and defined by the Marsh criteria. We decided to focus on the comparison of protein expression patterns among CD patients with different Marsh classification and controls. Patients were further grouped by preinfiltrative (Marsh 0), infiltrative plus infiltrative-hyperplastic (Marsh I -II), and destructive (Marsh III) lesion.

From the multigroup comparison, one of the most evident differences in protein expression was an increase in galectin-10 levels matching the increase of the Marsh index (one-way ANOVA $P = 0.0092$). Results are illustrated in Figures 1A and B. Galectin-10 is a lysophospholipase primarily expressed in eosinophils and basophils. It hydrolyzes lysophosphatidylcholine to glycerophosphocholine and a free fatty acid, and it is also known as Charcot-Leyden crystal protein and eosinophil lysophospholipase. On these grounds we decided to evaluate if there was a correlation between galectin-10 expression levels and the eosinophil number. For each patient, the number of eosinophils was assessed on H&E sections by a direct count with a light microscope (Fig. 2). Data obtained are reported in Table 2. Then, eosinophil numbers were linked to galectin-10-normalized spot volumes, as calculated by DeCyder software and shown in Table 2.

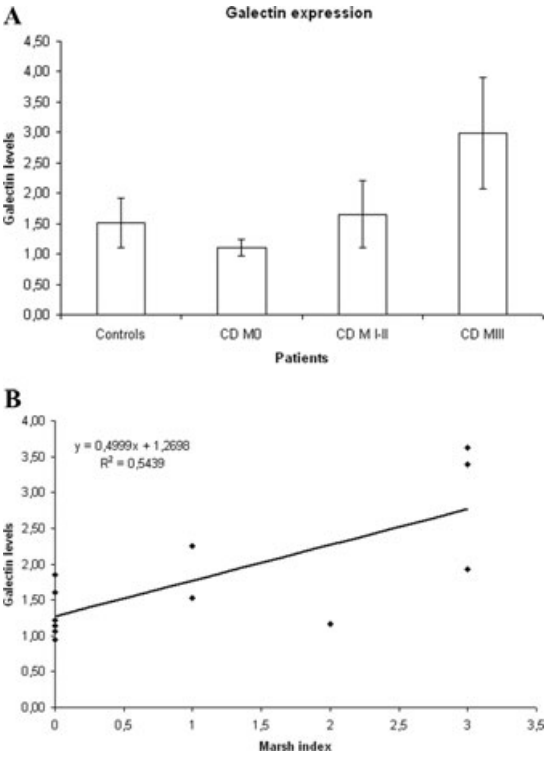

Figure 1. Expression level of galectin-10 is related to the extent of mucosal damage. (**A**) The galectin-10 level of expression was obtained as a volume ratio of cyanine-labeled galectin-10 spots of individual samples and normalized standard volume. Samples were grouped according to the Marsh histological grade, indicated as CD M0, I-II, and III, and controls. Averages (± SEM) of galectin-10 protein levels are from three independent gels. (**B**) Linear regression analysis generated by using the level of galectin-10 expression and Marsh grade; P value is 0.0092.

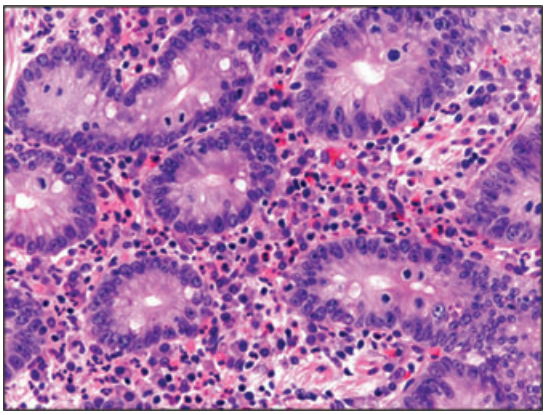

Figure 2. Eosinophil granulocyte infiltrate in the lamina propria. Duodenal biopsy section of a patient with a Marsh III CD (hematoxylin and eosin, original magnification, ×40), showing increased eosinophil granulocyte density suitable for quantitative assessment.

TABLE 2. Galectin-10 Expression Level and Number of Eosinophils of Each Sample

Patient	Galectin-10 normalized volume	Eosinophil number
Patient 1	3.63	20
Patient 2	1.93	4
Patient 3	3.40	ND
Patient 4	1.17	5
Patient 5	2.26	15
Patient 6	1.53	5
Patient 7	1.22	10
Patient 8	0.95	5
Patient 9	1.14	8
Patient 10	1.61	10
Patient 11	1.06	5
Patient 12	1.85	5

The linear regression statistical analysis evidences a very significant positive correlation between galectin-10 expression and the number of eosinophils found in the bioptic tissue (Fig. 3; $P = 0.0040$).

Discussion

By using the DIGE approach, we have identified an increase of galactin-10 expression in CD and have found that its expression was directly related to the histological grade of mucosal damage and to the number of eosinophils found in the duodenal lesion.

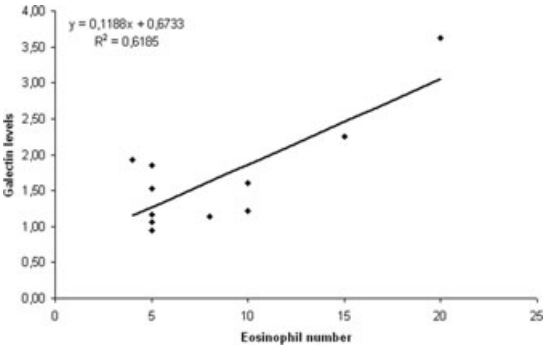

Figure 3. Expression level of galectin-10 is related to the eosinophil density. Linear regression analysis was generated by using the level of galectin-10 expression and the number of eosinophils; *P* value is 0.0040. The galectin-10 level of expression was obtained as a volume ratio of cyanine-labeled galectin-10 spots of individual samples and normalized standard volume. Number of eosinophils was calculated and recorded as the maximum number of eosinophils identified in each specimen in a high-power field.

DIGE is a powerful means for the analysis of differential protein expression in subgroups of samples. Nevertheless, it is generally accepted that to obtain quality genoma and proteoma analysis requires a homogeneous procedure for sample processing and that, in some cases, the method used may interfere with the obtained results. In particular, Foell *et al.*,[14] demonstrated that there is a specific downregulation of galectin-10 expression after a vital cryopreservation of the cells. However, the galectin-10 level alteration was evident only after addition of dimethylsulfoxide (DMSO) to the frozen medium, while the levels of galectin-10 in the fresh and frozen medium without DMSO remained unaltered. Additionally, we can now confirm that galectin-10 expression is maintained in frozen duodenal biotic tissues, even though from intestine high in proteolytic enzymes.

Galectin-10 is a lysophospholipase expressed primarily in eosinophils and basophils. It hydrolyzes lysophosphatidylcholine to glycerophosphocholine and a free fatty acid. It is

also known as Charcot-Leyden crystal protein and eosinophil lysophospholipase. It was previously found to be implicated in cancer, in allergy, in the hypereosinophilic syndrome, and in inflammatory bowel disease,[15–18] but for the first time it was found related to mild (Marsh II and IIIA) and still to more severe histological damage grade (Marsh IIIb and IIIc) CD.

An environmental factor, such as an infectious agent, is thought to precipitate the disease via various pathogenic mechanisms, such as molecular mimicry, resulting in modulation of the host's immune tolerance. There is evidence that CD is related to perinatal infections and that maternal milk may have a protective role. Observations imply that there is a relationship between viral infections, such as adenovirus 12 and hepatitis C virus, and the development of CD.[19]

The role of galectin-10 in pathological situations is still unknown as it has been poorly studied. In general, galectin-10 function is associated with the presence of eosinophils and with their degranulation. The mediators, released from eosinophil degranulation following their activation, are toxic both for parasites and host tissues. However, galectin-10 was proposed to have a host-protective role in some cases. In infants with the respiratory syncytial virus (RSV), a reduction of galectin-10 transcriptional level was evident in subjects with severe bronchiolitis with respect to children with only mild cold-like symptoms.[20] Since RSV infection is common but only a fraction of infants present severe respiratory manifestation, a genetic predisposition has been proposed. But, as the galectin-10 level and the number of eosinophils increase during bronchiolitis convalescence, a protective role of galectin-10 and eosinophil degranulation can be associated with downregulation of RSV inflammatory response.[20]

Galectin-10 has been implicated in sustaining inflammation in allergic conditions. In cases of allergic rhinitis,[22] galectin-10 expression has been found in both donors and patients only in the pollen season, but the level of galectin-10 and the number of eosinophils are much higher in allergic patients compared to controls. Moreover, in the patients, posttranslational alteration of the galectin-10 protein could cause the long-life duration of the protein in the mucosa. Similarly, galectin-10 mRNA is overexpressed in the case of aspirin-induced asthma,[23] suggesting galectin-10 to be a novel candidate marker for mucosal inflammation in allergic patients.

The role of galectin-10 and eosinophils in cancer immune response is less clear; nonetheless, current research indicates that the galectin family plays an important role in tumor response.[24] The propensity of eosinophils to secrete their granule proteins in patients with renal cell adenocarcinoma has led to the suggestion that eosinophils in cancer patients could have an enhanced capacity to kill cancer cells.[25] These results, by analogy with the cytotoxic activity shown in the course of infection, suggest that eosinophil degranulation may be toxic to the epithelium. Eosinophil degranulation was found to involve an increase in the permeability of the cell membrane when the internal potential is negative, as typically found in cancer cells. A carcinoma with a focused eosinophil accumulation and Charcot-Leyden crystals in prostate was described with eosinophils surrounding the carcinomatous focus and attacking the carcinoma cells;[26] crystals of galectin-10 were also found in pancreatic tumor,[27] in Hodgkin's lymphoma,[28] and in advanced-stage gastric carcinoma.[29] Overall data indicate that, in tumor stroma of a wide variety of epithelial neoplasms, eosinophils have generally been associated with an improved prognosis.[30–32]

Emerging evidence also suggests a role of eosinophils in the regulation of adaptive T cell immune response; eosinophils can promote either a Th2- or a Th1-immune response depending on the cytokines present in the microenvironment[33,34] and they are able to present antigen to T cells. Moreover, recently galectin-10 has been proposed as a novel marker for CD4+CD25+ regulatory T cell (Tregs) functions as it has implications in the energy and suppressive function of these cells.[35]

Eosinophil accumulation in the gastrointestinal tract is a common feature of numerous eosinophil-associated gastrointestinal inflammatory disorders, including food allergy, eosinophilic esophagitis, eosinophilic gastroenteritis, allergic colitis, and inflammatory bowel disease (ulcerative colitis and Crohn's disease),[15,36] and several clinical investigations suggest an important role for eosinophils in the etiology of these diseases. Indeed, a strong correlation has been demonstrated among clinical symptoms, disease severity, and increased numbers of this cell type in the gastrointestinal tract.[15,36,37] Moreover, recent experimental investigations have provided evidence for a role of eosinophils in gastrointestinal pathologies.[16–18,38–43]

An imbalance in the control of the reciprocal negative Th1/Th2 feedback loop is implicated in the pathogenesis of several autoimmune diseases and in the stimulation of B cells to produce antibodies,[34,35] findings also typically found in CD that suggest a possible role of eosinophils in celiac disease pathogenesis.

In conclusion, in this study we demonstrated for the first time a direct relation between the levels of galectin-10 protein accumulation and the grade of tissue damage in celiac disease. Moreover, we demonstrated a significant correlation of galectin-10 levels and the number of eosinophils found in bioptic tissue. Eosinophils are implicated in the initiation or potentiation of inflammatory reactions through the release of a range of inflammatory cytokines, chemokines, and lipid mediators. They contribute to innate and adaptive immune response and accumulate proportionally with the severity of several intestinal diseases. Consequently, although the pathological role of eosinophils and galectin-10 in CD is not understood, our results suggest that galectin-10 is a novel marker for evaluating CD tissue damage and that eosinophils could be a target for therapeutic approaches in the treatment of severe CD damage. Finally, our data provide insights into alterations associated with CD tissue damage and with CD pathogenesis.

Acknowledgments

The authors wish to thank the Centro di Biomedicina Molecolare (CBM), which provided the proteomic instruments, and Mrs. Anna Vallerugo for her writing assistance. The study was partially supported by Programma Integrato Oncologia, Tematica and Alleanza Contro Il Cancro (DM 21 luglio 2006, Art. 3).

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Green, P.H. & C. Cellier. 2007. Celiac disease. *N. Engl. J. Med.* **357:** 1731–1743.
2. Briani, C., D. Samaroo & A. Alaedini. 2008. Celiac disease: from gluten to autoimmunity. *Autoimmun. Rev.* **7:** 644–650.
3. Shaoul, R. & A. Lerner 2007. Associated autoantibodies in celiac disease. *Autoimmun. Rev.* **6:** 559–565.
4. Neuhausen, S.L. *et al*. 2008. Co-occurrence of celiac disease and other autoimmune diseases in celiacs and their first-degree relatives. *J. Autoimmun.* **31:** 160–165.
5. Bagnasco, M., L. Grassia & G. Pesce. 2007. The management of the patient with unexpected autoantibody positivity. *Autoimmun. Rev.* **6:** 347–353.
6. Tommasini, A. *et al*. 2004. Mass screening for coeliac disease using anti-human transglutaminase antibody assay. *Arch. Dis. Child.* **89:** 512–515.
7. Freitag, T. *et al*. 2004. The role of the immune response against tissue transglutaminase in the pathogenesis of coeliac disease. *Autoimmun. Rev.* **3:** 13–20.
8. Reif, S. & A. Lerner. 2004. Tissue transglutaminase–the key player in celiac disease: a review. *Autoimmun. Rev.* **3:** 40–45.
9. Oberhuber, G. *et al*. 1999. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur. J. Gastroenterol. Hepatol.* **11:** 1185–1194.
10. De Re, V. *et al*. 2007. Genetic insights into the disease mechanisms of type II mixed cryoglobulinemia induced by hepatitis C virus. *Dig. Liver. Dis.* **39:** S65–S71.
11. De Re, V. *et al*. 2007. Proteins specifically hyperexpressed in a coeliac disease patient with aberrant T cells. *Clin. Exp. Immunol.* **148:** 402–409.
12. De Re, V. *et al*. 2008. HCV inhibits antigen processing and presentation and induces oxidative stress

response in gastric mucosa. *Proteomics-Clinical Applications* **9:** 1290–1299.
13. De Re, V. *et al*. 2006. Type II. mixed cryoglobulinaemia as an oligo rather than a mono B-cell disorder: evidence from GeneScan and MALDI-TOF analyses. *Rheumatology* **45:** 685–693.
14. Foell, J.L. *et al*. 2008. Loss of detectability of Charcot-Leyden crystal protein transcripts in blood cells after treatment with dimethyl sulfoxide. *J. Immunol. Methods* **339:** 99–103.
15. Sampson, H.A. 1999. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J. Allergy Clin. Immunol.* **103:** 717–728.
16. Hogan, S.P. *et al*. 2008. Eosinophils: biological properties and role in health and disease. *Clin. Exp. Allergy* **38:** 709–750.
17. Hogan, S.P. & M.E. Rothenberg. 2006. Eosinophil function in eosinophil-associated gastrointestinal disorders. *Curr. Allergy Asthma Rep.* **6:** 65–71.
18. Wedemeyer, J. & K. Vosskuhl. 2008. Role of gastrointestinal eosinophils in inflammatory bowel disease and intestinal tumours. *Best Pract. Res. Clin. Gastroenterol.* **22:** 537–549.
19. Plot, L. & H. Amital. 2008. Infectious associations of Celiac disease. *Autoimmun. Rev.* [Epub ahead of print.] doi:10.1016/j.autrev.2008.10.001.
20. Fjaerli, H.O. *et al*. 2006. Whole blood gene expression in infants with respiratory syncytial virus bronchiolitis. *BMC Infect. Dis.* **6:** 175.
21. Fjaeri, H. *et al.* 2006. Whole blood gene expression in infants with respiratory syncytial virus bronchiolitis. *BMC Infect. Dis.* **6:** 175–182.
22. Ghafouri, B.K. *et al*. 2006. Comparative proteomics of nasal fluid in seasonal allergic rhinitis. *J. Proteome Res.* **5:** 330–338.
23. Devouassoux, G. *et al*. 2008. Galectin-10 mRNA is overexpressed in peripheral blood of aspirin-induced asthma. *Allergy* **63:** 125–131.
24. Liu, F.T. & G.A. Rabinovich. 2005. Galectins as modulators of tumour progression. *Nat. Rev. Cancer* **5:** 29–41.
25. Trulson, A. *et al*. 1997. The eosinophil granule proteins in serum, but not the oxidative metabolism of the blood eosinophils, are increased in cancer. *Br. J. Haematol.* **98:** 312–314.
26. Aydin, O. 2006. Charcot-Leyden crystals in a prostatic adenocarcinoma. *Diagn. Pathol.* **1:** 26.
27. Dvorak, A.M. *et al*. 1990. Ultrastructural localization of Charcot-Leyden crystal protein (lysophospholipase) to intracytoplasmic crystals in tumor cells of primary solid and papillary epithelial neoplasm of the pancreas. *Lab. Invest.* **62:** 608–615.
28. Axdorph, U. *et al*. 2001. Tissue eosinophilia in relation to immunopathological and clinical characteristics in Hodgkin's disease. *Leuk. Lymphoma* **42:** 1055–1065.
29. Caruso, R.A. *et al*. 2005. Degranulation patterns of eosinophils in advanced gastric carcinoma: an electron microscopic study. *Ultrastruct. Pathol.* **29:** 29–36.
30. Lotfi, R. *et al*. 2007. Eosinophilic granulocytes and damage-associated molecular pattern molecules (DAMPs): role in the inflammatory response within tumors. *J. Immunother.* **30:** 16–28.
31. Shunyakov, L. *et al*. 2004. The influence of host response on colorectal cancer prognosis. *Clin. Colorectal Cancer* **4:** 38–45.
32. Samoszuk, M. 1997. Eosinophils and human cancer. *Histol. Histopathol.* **12:** 807–812.
33. Liu, L.Y. *et al*. 2007. Generation of Th1 and Th2 chemokines by human eosinophils: evidence for a critical role of TNF-alpha. *J. Immunol.* **179:** 4840–4848.
34. Spencer, L.A. *et al*. 2008. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. *J. Leukoc. Biol.* [Epub ahead of print.]
35. Shi, H.Z. 2004. Eosinophils function as antigen-presenting cells. *J. Leukoc. Biol.* **76:** 520–527.
36. Furuta, G.T. *et al*. 1995. The role of the eosinophil in gastrointestinal diseases. *Curr. Opin. Gastroenterol.* **11:** 541–547.
37. Noel, R.J. *et al*. 2004. Clinical and immunopathologic effects of swallowed fluticasone for eosinophilic esophagitis. *Clin. Gastroenterol. Hepatol.* **2:** 568–575.
38. Forbes, E. *et al*. 2008. IL-9- and mast cell–mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J. Exp. Med.* **205:** 897–913.
39. Forbes, E. *et al*. 2006. ICAM-1-dependent pathways regulate colonic eosinophilic inflammation. *J. Leukoc. Biol.* **80:** 330–341.
40. Hogan, S.P. *et al*. 2002. Experimental analysis of eosinophil-associated gastrointestinal diseases. *Curr. Opin. Allergy. Clin. Immunol.* **2:** 239–248.
41. Rothenberg, M.E. & S.P. Hogan. 2006. The eosinophil. *Annu. Rev. Immunol.* **24:** 147–174.
42. Rothenberg, M.E. *et al*. 2001. Gastrointestinal eosinophils. *Immunol. Rev.* **179:** 139–155.
43. Simon, P. *et al*. 2000. A critical role for eotaxin in experimental oral antigen-induced eosinophilic gastrointestinal allergy. *Proc. Natl. Acad. Sci. USA* **97:** 6681–6686.