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Focal-Enhanced Gastritis in Regressive Autism with Features Distinct from Crohn's and *Helicobacter Pylori* Gastritis

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BACKGROUND: Immunohistochemistry allowed recent recognition of a distinct focal gastritis in Crohn's disease. Following reports of lymphocytic colitis and small bowel enteropathy in children with regressive autism, we aimed to see whether similar changes were seen in the stomach. We thus studied gastric antral biopsies in 25 affected children, in comparison to 10 with Crohn's disease, 10 with *Helicobacter pylori* infection, and 10 histologically normal controls. All autistic, Crohn's, and normal patients were *H. pylori* negative.

METHODS: Snap-frozen antral biopsies were stained for CD3, CD4, CD8, $\gamma\delta$ T cells, HLA-DR, IgG, heparan sulphate proteoglycan, IgM, IgA, and C1q. Cell proliferation was assessed with Ki67.

RESULTS: Distinct patterns of gastritis were seen in the disease states: diffuse, predominantly CD4+ infiltration in *H. pylori*, and focal-enhanced gastritis in Crohn's disease and autism, the latter distinguished by striking dominance of CD8+ cells, together with increased intraepithelial lymphocytes in surface, foveolar and glandular epithelium. Proliferation of foveolar epithelium was similarly increased in autism, Crohn's disease and *H. pylori* compared to controls. A striking finding, seen only in 20/25 autistic children, was colocalized deposition of IgG and C1q on the subepithelial basement membrane and the surface epithelium.

CONCLUSIONS: These findings demonstrate a focal CD8-dominated gastritis in autistic children, with novel features. The lesion is distinct from the recently recognized focal gastritis of Crohn's disease, which is not CD8-dominated. As in the small intestine, there is epithelial deposition of IgG.

BACKGROUND

Recent reports from two groups have suggested that lymphocytic colitis and small bowel enteropathy occur in children with regressive autism (1–5). The findings have been endoscopically and histologically subtle (1, 5), but investigations such as immunohistochemistry and disaccharidase quantitation have confirmed significant abnormality (2–5). In both colon and duodenum, epithelial damage appeared prominent, with pericellular aggregation of CD8 T cells (2, 3). In the duodenum, there was focal deposition of IgG and complement C1q on the basolateral enterocyte membrane in 23/25 children studied, not seen in disease controls (3). It is currently uncertain whether this represents a true autoimmune phenomenon, or a nonspecific consequence of a lymphocyte-dominated inflammatory response. It is also uncertain whether such intestinal involvement is a previously unrecognized component of classic autism, or a specific feature limited to a subgroup of children with a regressive phenotype and prominent gastrointestinal symptoms. The autistic spectrum includes several disorders, with common areas of disturbance in social interaction, communication, and behavioral flexibility but with wide variation in IQ, ranging from normal or high in Asperger syndrome to severely impaired in other cases (6). Several distinct genetic disorders may also present with similar autistic features. In some cases the earliest signs can be seen in the first year of life, while other children show a later loss of previously acquired skills with the onset of behavioral abnormalities, with no abnormalities having been recognized in the first year (regressive autism).

The pathology of gastritis is often subtle, and recognition of distinct lesions has required the initial use of special techniques, before pattern recognition is established to allow subsequent diagnosis by straightforward histology. While the diagnosis of *Helicobacter pylori* (HP) is now routine, this was not the case for some time after its initial report (6). Similarly, the potentially diagnostic focal gastritis of Crohn's disease was only recognized after immunohistochemical assessment (7–9), but is now recognized as an emerging diagnostic entity (10). Gastritis was reported in 15/36 children with autistic spectrum disorders by Horvath *et al.*, although no distinctive features could be recognized on conventional staining

Table 1. Clinical Details of the Patients Studied

	No.	M:F	Age (yr)	Hb (g/dl)	ESR (mm/h)	CRP (mg/dl)	Albumin (g/l)	IgA (g/l)
Autistic children	25	20:5	6.2 (0.4)	12.3 (1.4)	5 (4)	1 (1)	42.0 (4.5)	0.9 (0.5)
Normal controls	10	4:6	8.5 (1.4)	12.0 (1.5)	7 (3)	1 (0.5)	41.0 (3.0)	1.1 (0.7)
H. pylori infection	10	7:3	11.9 (0.8)	12.7 (4.3)	6 (4)	<1	43.0 (3.0)	2.4 (1.5)
Crohn's disease	10	5:5	11.5 (0.9)	10.4 (3.1)	35 (66)	22 (64)	34.5 (10.0)	2.1 (2.7)

All figures represent medians with interquartile range in parentheses.

(4). As the duodenal and colonic lesion in autism is dominated by infiltration of T lymphocytes, particularly CD8 cytotoxic/suppressor cells, we investigated the hypothesis that this gastric lesion may show similar characteristics by extensive immunohistochemical study of antral biopsies in 25 affected children, making comparison to Crohn's disease, histologically normal controls, and HP gastritis. We report findings of a novel and distinct form of gastritis in children with regressive autism, characterized by CD8 lymphocyte infiltration, increased epithelial proliferation, and colocalization of IgG and complement C1q on the surface epithelium and its basement membrane.

PATIENTS AND METHODS

Clinical details of all the patients involved in the study are given in Table 1. The autistic children in this study had been referred for gastroenterological assessment by either consultant pediatricians (11/25 cases) or general practitioners (14 cases) because of gastrointestinal symptoms, such as constipation and/or diarrhea, abdominal pain, and distension. All cases had received a diagnosis of autistic spectrum disorder at median age 3 yr (range: 1–5) from developmental pediatricians and/or consultant child psychiatrists, and all remained under follow-up by developmental pediatricians with a current diagnosis of autistic spectrum disorder. Thirteen cases had been referred to regional or tertiary centers, where the diagnosis had been confirmed, with eight patients initially investigated by consultant pediatric neurologists for possible degenerative neurological disorders and two were seen by consultant geneticists. Twenty three children had been diagnosed with core autism by DSM-IV criteria, one as autistic spectrum by Diagnostic Interview for Social and Communication Disorders but atypical autism by DSM-IV criteria, and one as attention deficit with hyperactivity disorder secondary to Asperger syndrome. There had been developmental concerns in the first year in six cases (motor delay in all, impaired hearing in one, abnormal socialization in two, possible convulsions in one). In 2/25 cases, diagnosis was first suggested around 1 yr of age, one associated with chromosomal disorder (mosaic 18q deletion), and in five further cases there was failure to develop language in the second year of life, together with evidence of impaired socialization and absence of imaginative play, but without any clear episode of regression. In 18/25 cases, development had appeared normal throughout the first year apart from mild motor delay in one case, and there was apparently normal subsequent linguistic and social development before a regression at median age 18 months (range: 15–30), characterized by loss of language or echolalia (18/18 cases) together with avoidance of eye contact (13/18), abnormal socialization (14/18), and play (14/18) or hyperactivity (6/18). Epilepsy developed in 2/25 children, who were receiving anticonvulsant therapy at the time of investigation, while celiac disease had been identified in one case. A history of recurrent otitis media was reported in seven children in the first year of life, and eight children later required adenotonsillectomy or grommet insertion. One child had required neonatal cardiac surgery for transposition of the great vessels. At the time of investigation, 15 children were currently assessed by their developmental pediatricians as demonstrating moderate or severe global developmental delay, six as mild developmental delay, and four as within normal developmental limits. Five children were attending mainstream education, of whom three required 1:1 teaching on a part-time basis, while 17 were attending special schools. MRI examinations had been performed in nine children prior to referral and reported normal. Twelve of the children were on cow's milk and/or wheat exclusion diets at the time of referral and endoscopy.

The autistic patients were selected for the study simply on the basis of having undergone upper gastrointestinal endoscopy and having frozen gastric biopsies available for staining. We studied the most recent 25 children in whom snap-frozen gastric biopsies were available and did not exclude any patients from the study. Duodenal findings in two of these patients have been reported (3). The controls studied were children investigated to exclude possible gastroesophageal reflux or enteropathy, when endoscopic findings were normal and all histology had been routinely reported within normal limits. We also included 10 patients with newly diagnosed Crohn's disease, who had undergone upper endoscopy as part of initial diagnostic assessement, and 10 patients with active HP positive gastritis, confirmed by both CLO test and histology. The biopsies were taken according to routine unit protocol, with one antral biopsy sent for histology, and one used for CLO test, with additional histological specimens taken only when there was endoscopic abnormality elsewhere in the stomach. Additional frozen biopsies were obtained from all the children, with informed parental consent, as approved by the local Research Ethics Committee. Formal blinded assessment of H&E stained formalin-fixed biopsies, taken from adjacent antral mucosa, was performed, with diagnosis of gastritis made using Updated Sydney system criteria (11) by AA.

Immunohistochemical Analysis

Biopsies were taken endoscopically from the gastric antrum, using a Fujinon EG410HR endoscope with 2.8 mm biopsy channel and standard forceps, and immediately snap-frozen. Biotin/avidin immunohistochemistry (Vectastain Elite, Vector Laboratories, Peterborough, UK) was used for peroxidase immunohistochemistry as previously described (2, 3). Primary antibodies included T-cell markers CD3 (dilution 1:20), CD4 (1:20), CD8 (1:25), the proliferation marker Ki67, HLA-DR (1:50), all from Dako UK, and $\gamma\delta$ T cells (1:25, T cell Sciences, USA). Basement membrane thickness was assessed with heparan sulphate proteoglycan (1:50, Seikagaku, UK). FITC-conjugated antihuman immunoglobulin antibodies were used to study the distribution of IgA, IgG, and IgM within the mucosa (1:40, Dako), while complement C1q was localized with a rabbit polyclonal antibody (1:200, Dako) followed by TRITC-conjugated swine antirabbit antibody.

Quantitation

All quantitations were performed in strictly blinded fashion by FT or SHM. Reproducibility of quantitation was formally assessed and was within ±8%. Intraepithelial T lymphocyte (IEL) density and Ki67 positive cells were determined per 100 epithelial cells by manual counting. The density of subepithelial CD3 and lamina propria CD4 lymphocytes were determined semiquantitatively, as previously reported by Oberhuber *et al.* (7) as follows: 0, no labeled cells; 1, up to 10 labeled cells per high-power field (HPF); 2, 11–40 labeled cells/HPF; 3, more than 40 labeled cells/HPF. Whether the infiltrate was focal or diffuse was noted. We assessed mucosal CD8 infiltration in glandular epithelium by determining the percentage of glands containing two or more intraepithelial and three or more periglandular CD8 cells, taking a mean from at least three HPFs. The epithelial and lamina propria expression of heparan sulphate proteoglycan (HSPG), was assessed semiquantitatively on a scale from 0 (absent) through 3 (normal) to 4 (enhanced expression) as previously reported (3).

Statistical Analysis

Data are expressed as median with interquartile range except when stated. Comparison was made between the autistic group and the other groups using the Kruskal-Wallis test, with individual comparisons then made between the autistic group and controls, children with Crohn's disease and the HP group using the Mann-Whitney U test. A p-value of less than 0.05 was considered significant.

RESULTS

As shown in Table 1, the Crohn's patients showed significantly raised inflammatory markers compared to the autistic patients ($p < 0.01$ for ESR, $p < 0.05$ for CRP), with lower hemoglobin ($p < 0.05$) and albumin ($p < 0.05$). IgA concentrations in the autistic children were lower than the Crohn's disease and HP groups ($p < 0.05$). Endoscopic findings were relatively nonspecific in all groups apart from the children with HP. Antral erythema without ulceration was noted in 1/10 control, 4/10 children with Crohn's disease, and 5/25 autistic children. In some autistic cases a pale, edematous antral mucosa was seen, but most appeared endoscopically normal. Formal blinded histological assessment confirmed 8/10 controls as normal, while two had minimal increase in chronic inflammatory cells. In the HP group, 5/10 showed severe chronic diffuse gastritis, 2/10 moderate, and 3/10 mild changes. In the Crohn's disease group 3/10 children had severe active gastritis, 5/10 mild/moderate chronic focal gastritis, and 2/10 mild diffuse gastritis. Among the autistic children, 12/25 biopsies were within normal limits, 8/25 showed mild focal chronic gastritis, 3 moderate focal gastritis, and 2/25 mild diffuse gastritis. Lymphocytic aggregates were seen in 5/10 Crohn's patients, 3/10 patients with HP gastritis, 7/25 autistics, and no controls, while glandular disruption was seen in eight Crohn's, seven HP, and six autistic children.

Immunohistochemistry

Immunohistochemistry showed clear differences between the autistic group and the other groups (Figs. 1–3). No differences were noted between those on exclusion diets and those with unrestricted diets for any parameter. Four specimens (two autism, one normal, and one HP showed apparent gastric body mucosa, with findings similar to the group's antral biopsies in each case). IEL density was increased in both surface and glandular epithelium in autism (Figs. 1 and 2), with striking predominance of CD8+ but not $\gamma\delta$ T cells. In surface epithelium, the mean density of CD8+ IEL in the autistic children (24 per 100 epithelial cells) was approximately double that seen in the other groups (Fig. 2). Within the lamina propria, minimal infiltration of CD3 T cells was seen in 7/10 controls, and similar normal findings were identified in 3/10 Crohn's disease and 4/25 autism but in no HP specimens. Subepithelial CD3 cell density in autism (median score: 2, range: 1–3) was increased above controls (1, 1–2, $p < 0.05$), but similar to Crohn's disease (3, 1–3, $p > 0.05$) and lower than in HP infection (3, 1–3, $p < 0.05$). Two patterns of increased lamina propria T-cell infiltration were seen (Fig. 1). Diffuse infiltration was seen in 6/10 with HP, 4/10 with Crohn's disease, and 7/25 with autism compared to 1/10 controls (Fig. 2). The density of CD4 infiltration was higher in the autistic children (median score: 2, range: 1–3) than in controls (1, 1–2, $p < 0.01$), but lower than in both Crohn's disease (3, 1–3, $p < 0.01$) and HP infection (3, 1–3, $p < 0.05$). Focal-enhanced gastritis, with aggregates of CD3 cells around foveolar or glandular epithelium, was seen in 6/10 with Crohn's disease and 19/25 with autism compared to 3/10 with HP and 2/10 controls (who showed single foci). Distinct patterns of CD4 and CD8 cells were seen, with CD4+ infiltration substantially greater than CD8+ in the HP cases, while in the focal aggregates seen in the normal and Crohn's cases, CD4+ and CD8+ cells were approximately equal. By contrast, in the 19 autistic

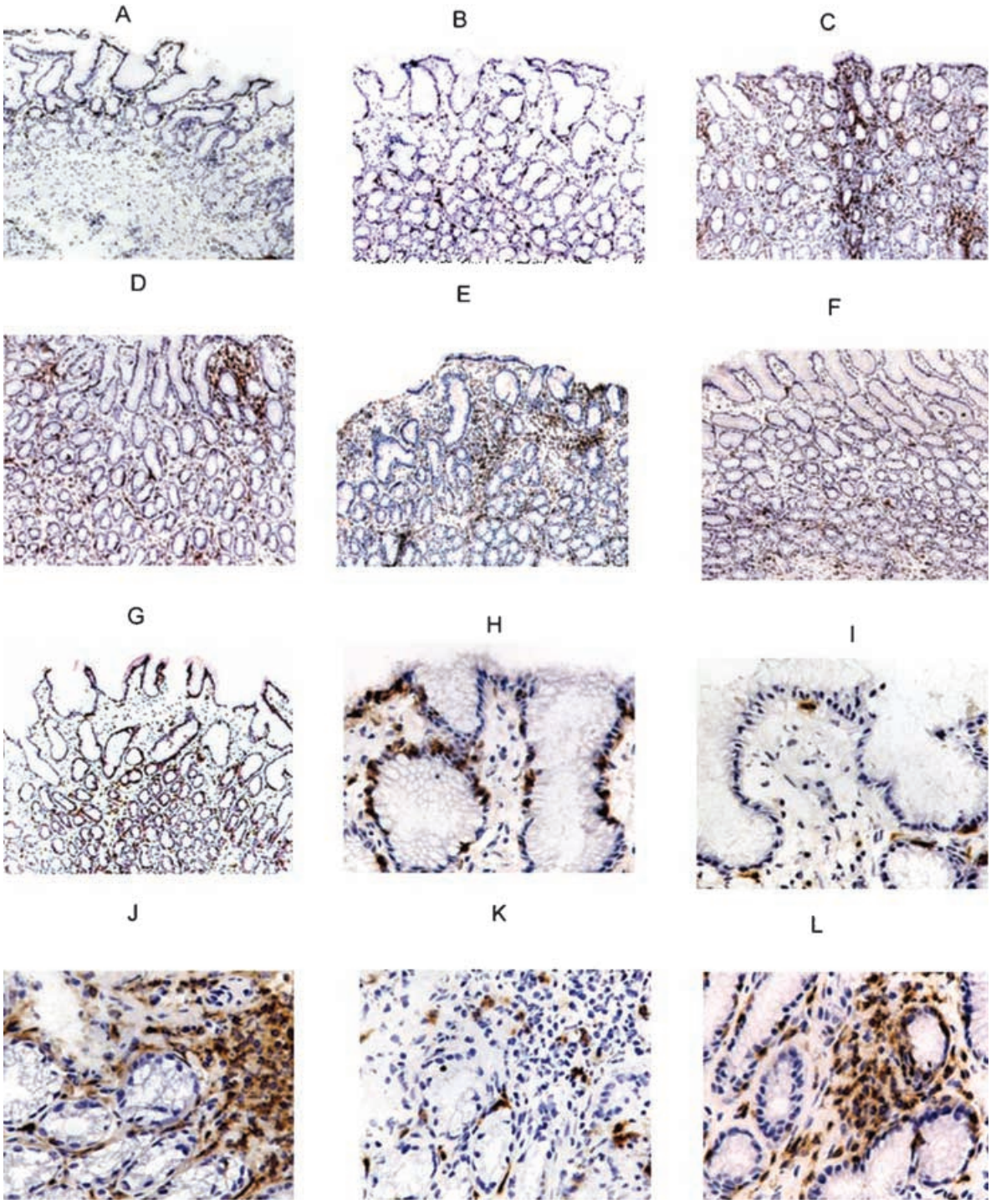

Figure 1. Immunohistochemical localization of mucosal lymphocytes in antral biopsies. Immunoreactive cells are stained brown. Biopsies from four autistic children are shown. (*A*) CD3 cells in a normal control, showing a low density of scattered T cells and few intraepithelial lymphocytes (original magnification ×10). (*B*) CD3 cells in Crohn's disease, showing focal increase in T cell density, but without significant numbers of intraepithelial lymphocytes (×10). (*C*) CD3 cells in *H. pylori* infection, with diffuse increase in mucosal T cell density in addition to focal aggregates (×10). (*D*) CD3 cells in autism, showing increased density of mucosal T cells together with focal-enhanced gastritis–dense aggregations of T cells around glandular and foveolar epithelium (×10). (*E*) CD4 cell distribution in Crohn's disease, showing focal aggregates in the lamina propria (×10). (*F*) CD4 cell distribution in an autistic child, with minimal evidence of the aggregations seen in Crohn's disease (×10). (*G*) Contrasting findings of dense CD8 infiltration in surface and glandular epithelium in an autistic child (×10). This was not seen in the other conditions. (*H* and *I*) High-power views of surface epithelial CD8 infiltration in (*H*) an autistic child and (*I*) a child with *H. pylori* infection (×40). (*J–L*) The distinct features of focal-enhanced gastritis in Crohn's disease and autism. A focal periglandular aggregate of CD3 T cells in Crohn's disease (*J*) shows relatively few CD8 cells on serial section (*K*). By contrast, CD8 staining of a focal periglandular lesion in autism (*L*) shows dense clustering of CD8 cells.

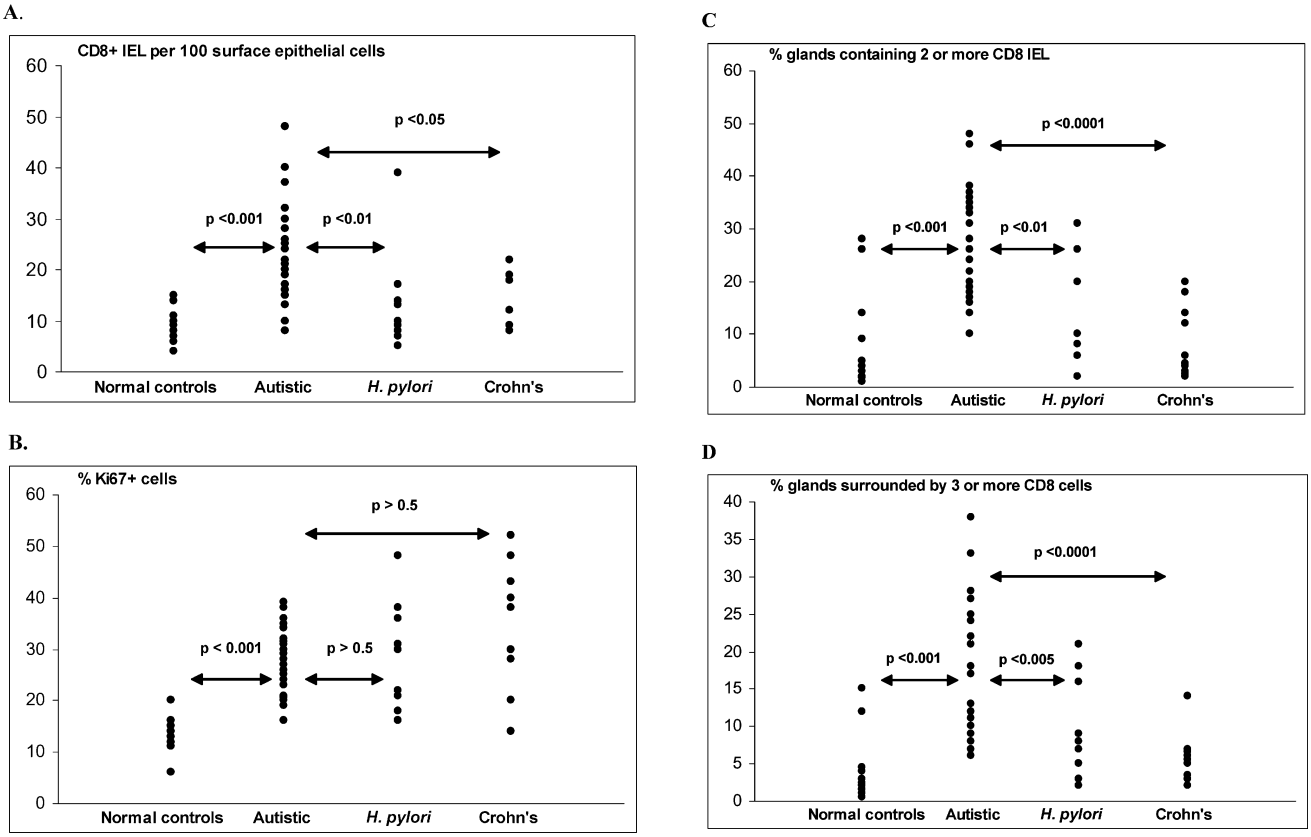

Figure 2. Quantitation of CD8+ lymphocytes and Ki67+ cells in individual cases. Comparison between groups was made using the Kruskall-Wallis test. Each data point in (*C*) and (*D*) represents the mean from at least three high-power fields. (*A*) The density of CD8+ intraepithelial lymphocytes within surface epithelium. (*B*) The percentage of foveolar epithelial cells expressing the proliferative marker Ki67. (*C*) The percentage of antral glands infiltrated by two or more CD8+ intraepithelial lymphocytes. (*D*) The percentage of antral glands surrounded by three or more CD8+ T cells.

children with focal aggregates, CD8+ cells dominated over CD4 in all but five cases, and the density of periglandular and glandular intraepithelial CD8 cells was higher than all other groups (Fig. 2).

Ki67 expression was increased in both lamina propria lymphocytes and surface epithelium in the autistic, HP, and Crohn's disease groups (mean epithelial proliferation: 29–34%) compared to controls (13.5%, $p < 0.001$ for all disease groups, Fig. 2). HLA-DR expression was focal in the glandular epithelium in 10/25 autistic children, 1/10 controls, 1/10 HP, and 6/10 Crohn's patients, while surface epithelium was HLA-DR +ve in 2/25 autistic children, 0 controls, 4/10 with HP, and 3/10 with Crohn's disease. Focal degradation of basement membrane HSPG was seen in 11/25 autistics, 1/10 controls, 8/10 with HP, and 7/10 with Crohn's disease, while basement membrane thickening was noted in 13/25 autistics, 0 controls, 4/10 with HP, and 6/10 with Crohn's disease.

Immunoglogulin Deposition

Clear differences were seen between the groups in the pattern of IgG deposition. The most striking finding was the subepithelial basement membrane and pericellular epithelial deposition of IgG in the children with autism. This was seen in 20/25 autistics, with overall epithelial IgG density clearly above the other groups (Fig. 3). The 2/10 of Crohn's disease patients showed a patchy epithelial IgG deposition, but this was not seen in the basolateral membrane as it was in the autistic children, whereas dense IgG deposition was observed in the lamina propria of the HP group. In some autistic children the epithelial IgG deposition was patchy and less extensive than seen in the duodenum (3), while in others it was striking. On double-staining for complement C1q, a largely similar expression pattern was seen, strongest within the subepithelial basement membrane, and extensive colocalization was identified on double exposure.

DISCUSSION

These data confirm Horvath *et al.*'s initial report of gastritis in autistic children (4), and extend their findings to demonstrate a novel form of focal gastritis dominated by CD8+ T cells. The pattern of lymphocyte infiltration is more similar to that recently identified in Crohn's disease (7–10) than to the more florid diffuse infiltrate of HP infection, but shows reduced numbers of CD4+ cells compared to the Crohn's lesion (8)

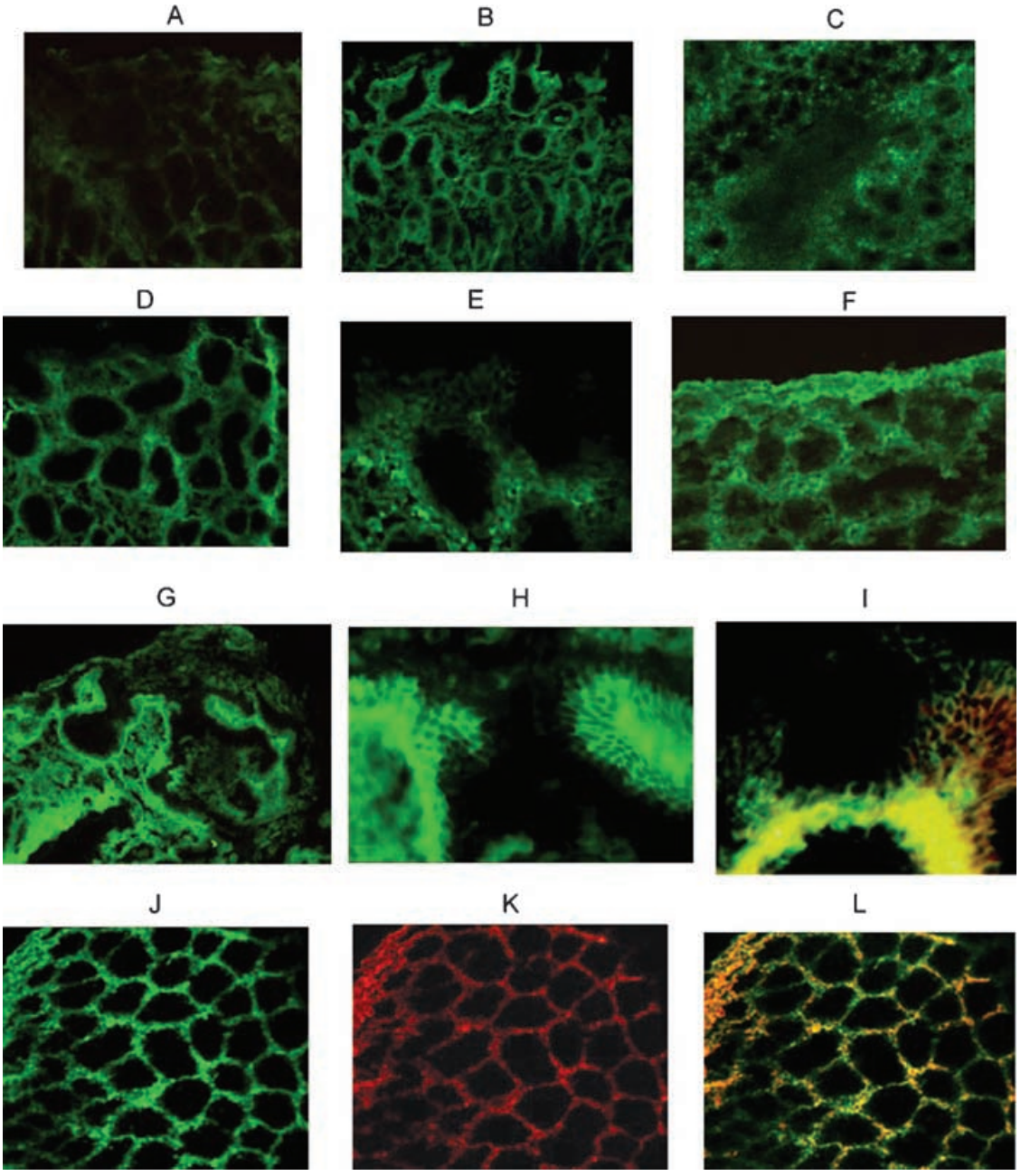

Figure 3. Immunofluorescence for IgG (FITC green) and C1q (TRITC red) in antral biopsies. Colocalized IgG and C1q (*I*, *L*) appears yellow. Biopsies from four autistic children are shown (none of whom were in Fig. 1). (*A*) Normal control, showing only minor IgG deposition (original magnification ×10). (*B*) *H. pylori* gastritis, with extensive IgG deposition in lamina propria and subepithelial basement membrane. Minimal IgG is bound to surface or glandular epithelium (×10). (*C*) IgG production within a large lymphoid follicle in *H. pylori* gastritis, but no deposition within adjacent glandular epithelium (×10). (*D*) Patchy focal IgG deposition in Crohn's gastritis, particularly around glandular epithelium. The surface epithelium is however normal (×10). (*E*) High-power view of focal perifoveolar IgG deposition in Crohn's disease (×40). The surface epithelium is unstained however. (*F*) Dense IgG deposition on surface epithelium in an autistic child (×10). (*G*) Strong IgG expression on surface epithelium and subepithelial basement membrane in an autistic child (×10). (*H*) High-power view of the surface epithelial IgG deposition in the biopsy shown in *G* (×40). (*I*) Colocalization of IgG and C1q on basement membrane and surface epithelium in another autistic child (×40). This was not seen in the other conditions. (*J–L*) IgG and complement C1q deposition in autistic child (×10). The surface epithelium lies at the top left. IgG FITC is shown in *J*, C1q TRITC in *K* and double exposure showing focal colocalization in *L*. Focal periglandular aggregates can be seen, and the surface epithelium shows strong colocalization.

and increased CD8+ cells. Two further features distinguish the lesion in the autistic children, a striking increase in intraepithelial lymphocyte infiltration, again CD8-dominated, involving glandular, foveolar, and surface epithelium, and deposition of IgG and complement C1q on surface epithelium. The features are in many ways concordant with those previously reported in the duodenum and colon (2, 3), with a histologically subtle but immunohistochemically distinct

CD8-dominated lesion targeted toward the epithelium. The significance of a CD8-dominated process with apparent epithelial tropism at several sites in autistic children is unknown, but may relate to an increased expression of chemokines by epithelium. CD8 cells may demonstrate cytotoxic or suppressive functions, and may also secrete a similar spectrum of cytokines to CD4 cells. Further specific analysis of lymphocyte reactivity is needed to determine the significance of this infiltration. These changes are however associated with marked disturbance of the enteric flora in the stomach, duodenum, and colon in regressive autism (12).

We acknowledge the limitations of studying single biopsies from one area of the stomach, which represented our clinical practice at the time, and thus used immunohistochemistry for characterization. However, in cases where gastric body mucosa was obtained similar focal infiltration was seen, and we have seen similar histological features in subsequent cases where gastric body biopsies were taken. There has been recent reassessment of optimal biopsy strategy in pediatric gastroscopy, and a more extensive biopsy protocol suggested, involving different regions of the stomach (13, 14). Study of multiple biopsies, taken with deep sampling forceps from different sites and examined by experienced gastrointestinal pathologists, should maximize the chances of identifying focal aggregations of lymphocytes or glandular disruption by routine histology. As focal lymphocytic gastritis is rare in childhood (13, 14), this may be a more specific histological feature than seen in duodenum or colon (2, 3). However, such findings in an individual child will have to be considered in the context of the overall gastroenterological assessment. Celiac disease should be excluded as a potential cause of lymphocytic gastritis (10), and we recommend that the serological testing is performed before wheat or gluten exclusion diets are commenced in any child.

The functional significance of this lesion remains uncertain. We have seen over-representation of children with a history of cognitive regression in those referred with gastroenterological symptoms (1–3), and it remains unclear whether gastrointestinal abnormalities may be more widely expressed in autistic spectrum disorders. The relevance and specificity of regression as a feature distinguishing valid subgroups in autism remains unclear. Recent data from the United States suggest a similar degree of genetic predisposition in regressive autism as in nonregressive disease, with an incidence of broader autism phenotype in parents in about 30% of cases (15), notably higher than in an extensive United Kingdom series (16). However, there were important phenotypic differences in the regressive group, who demonstrated less macrocephaly and congenital abnormality, suggesting that genetic predisposition may differ between regressive and nonregressive autism (15). In this study we identified similar findings in children with regressive and nonregressive histories, and suggest that further assessment of much larger cohorts, with collaboration between pediatric gastroenterologists and child psychiatrists, will be required to place these findings in context. However, we believe that this study provides important guidance to histopathologists for such future studies.

Assessments of gastrointestinal symptoms in autistic children have suggested that up to half of them have clinical evidence of gastrointestinal abnormality (17). Intriguingly, early postmortem examinations in adults with schizophrenia identified gastrointestinal and hepatic inflammation (18). However, it is important to recognize that autism and schizophrenia are distinct conditions (19), albeit with some shared susceptibility loci (20). We speculate that the cognitive abnormalities in some autistic children may represent the tip of an iceberg of poorly recognized systemic abnormality. The concept that genetically determined autism may show multisystem abnormalities is supported by findings of immunological and gastrointestinal abnormality in Rett syndrome (21, 22), in which overlapping features of developmental regression are associated with impaired control of gene expression through DNA methylation (23). There is also evidence of abnormal regulation of lymphocyte activation in children with autistic spectrum disorders, including surface marker expression suggesting partial activation (24) and increased secretion of the cytokine tumor necrosis factor-α (25). The other significant possibility is that these features represent an autoimmune response to a gut epithelial determinant, which may also be expressed within neural tissue, and we consider this an area worthy of further study. While the genetic predisposition to autistic spectrum disorders is undoubted, with identified chromosomal susceptibility loci and specific candidate genes (20, 26), there is also increasing evidence for a predisposition to autoimmunity (27). We have discussed this in more depth in recent papers (2, 3). As neurodevelopmental features in some strains of spontaneously autoimmune mice show some similarities to autism, with changes in neuronal cellularity and the presence of brain-reactive autoantibodies, and regression may be prevented in these animals by early immunosuppression (28), we believe it important to determine the relevance of this potentially autoimmune intestinal pathology.

The mucosal inflammation in children with autism, although histologically subtle, is panenteric, affecting colon, ileum, duodenum, and stomach (1–5). This may have therapeutic relevance as some affected children show responses to gut-targeted maneuvres such as vancomycin therapy (12, 29) or dietary exclusions (30). There are as yet only anecdotal reports of response to immune modulation (5), and we believe that the use of agents with potentially significant adverse effects should be limited to controlled clinical trials. We are concerned by the unregulated use of potentially toxic agents, often sold through the Internet, in such vulnerable cases. However, we also believe that standard treatment should not be denied a symptomatic child with confirmed pathology because he or she is autistic. For children with symptoms of abdominal pain or sleep disturbance found to have histologically confirmed gastritis or esophagitis, a therapeutic trial of conventional acid inhibitory therapy would appear clinically indicated, and it is probably advisable

for HP infection to be treated if identified. We also use aminosalicylates such as mesalazine in children who have histological evidence of significant duodenitis or colitis. We suggest that the pediatric gastroenterologist may play a valid role in the global assessment of the child with autism.

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Conflict of interest statement: We are not aware of any conflicts of interest for any of the authors. Franco Torrente and Paul Ashwood receive financial support from Visceral (http://www.visceral.org.uk/what.htm), but this study was conducted and written entirely independently.

Contributions: Franco Torrente performed the immunohistochemical analysis and quantitation, and was assisted by Paul Ashwood. Andrew Anthony performed the histological analysis, while Rob Heuschkel and Mike Thomson contributed to patient assessment, endoscopy, and specimen handling. Simon Murch supervised the staining and quantitation, peformed data analysis and wrote the first draft of the manuscript with Franco Torrente. All authors contributed to the final draft.

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