Plasma cell infiltration of the small bowel: lack of evidence for a non-secretory form of alpha-heavy chain disease

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SUMMARY Eight patients with diffuse plasma cell infiltration of the small bowel who had the clinical features of immunoproliferative small intestinal disease (IPSID), but whose serum was negative for free alpha-heavy chains, were investigated for evidence of a non-secretory form of alpha-chain disease (α-CD). Molecular sieving and immunoblotting of serum, immunoperoxidase staining of biopsy specimens, and in vitro protein synthesis studies utilising an immunoprecipitation technique and polyacrylamide gel electrophoresis, failed to detect any new cases of α-CD. Four of the eight cases were found to have diffuse intestinal lymphoma. The remaining four patients, who were unsuccessfully investigated for evidence of a significant abnormality in cellular immunity, have not developed detectable α-CD protein or lymphoma over a mean of 143 months. Despite continuing exposure to possible environmental stimuli, it is concluded that not all cases of IPSID elaborate detectable α-CD protein or evolve to lymphoma.

Immunoproliferative small intestinal disease (IPSID) invariably presents with a malabsorption syndrome resulting from a diffuse and extensive, predominantly plasma cell infiltration of the bowel.¹ Most reported cases have originated from the Mediterranean region, Middle East, Iran, Iraq, Indian subcontinent and South Africa. The IPSID spectrum of disorders includes malignant conditions such as immunoblastic (Mediterranean-type) lymphoma, alpha-chain disease (α-CD) with or without associated lymphoma, and ‘benign’ conditions characterised by malabsorption with a dense lymphoplasmacytic infiltration of the bowel in patients without lymphoma, and in whom alpha-chains cannot be identified.²

It has been postulated that diffuse intestinal plasma cell infiltration resulting from possible antigenic stimulation leads in susceptible individuals to the eventual production of α-CD protein;³ and this process may ultimately evolve to lymphoma.⁴ Although α-CD protein has been found in the serum in 20–64% of cases of IPSID,⁵ ⁶ it has been suggested that the vast majority of cases of IPSID are in fact α-CD.⁷ The recent report of a non-secretory form of α-CD⁸ has prompted a re-evaluation of the prevalence of α-CD in IPSID patients.

We have investigated patients with diffuse plasma cell infiltration (PCI) of the bowel in order to determine whether patients with PCI and malabsorption, but without evidence of IPSID-associated lymphoma (IAL) or free alpha-chains in their serum,⁹ have a non-secretory form of α-CD. We have determined whether these patients have a deficit in cell-mediated immunity, as has been suggested,¹ ¹⁰ and whether all patients with IAL have evidence of α-CD.

Methods

Patients

Eleven patients (seven women, four men) were studied. All were screened at presentation and at six monthly intervals throughout their course for the presence of free alpha-heavy chains in the serum by a modification of the immunoselection technique of
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Radl. The main clinical data for each patient at presentation are shown in Table 1. Three patients had confirmed α-CD; one (GW) was untreated while another (TC) was in partial remission and asymptomatic with virtually normal histology. The third (CC) was in full remission and off all treatment for eight years. Four subjects had IAL without demonstrable α-CD protein in the serum. The remaining four patients had a dense plasma cell infiltrate of the bowel, and at the time of study had been followed for a mean of 9-2 years without the development of lymphoma or overt α-CD. Over an additional 2-7 year period, patients with α-CD and IAL were recruited into the study, but no change in status of the PCI subgroup (follow up period 11-9 years) was observed. Ten of the 11 patients (90%) had biochemical evidence of malabsorption, and intestinal parasitic infestation was found in six (54%).

BIOPSIES
Endoscopically obtained distal duodenal/jejunal biopsies were transported to the laboratory in buffered formalin and fixed for light microscopy. Villous architecture and cellular infiltrate was assessed and the presence of IgA, IgG, IgM and kappa and lambda chains evaluated by standard immunoperoxidase (PAP) techniques. Other specimens were taken for homogenisation and tissue culture (see below).

PROTEIN STUDIES
Routine protein and immunoglobulin electrophoresis on serum was done according to standard techniques. The immunoselection plate method of Radl was used to detect the presence of free alpha-chains in serum, preserved jejunal juice, concentrated urine and biopsy homogenates. The

<table>
<thead>
<tr>
<th>Table 1 Presenting features</th>
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<tbody>
<tr>
<td><strong>Symptoms and signs</strong></td>
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<tr>
<td>Diarrhoea, weight loss</td>
</tr>
<tr>
<td>Diarrhoea, weight loss, vomiting, abdominal pain</td>
</tr>
<tr>
<td>Diarrhoea, pain, weight loss, abdominal mass</td>
</tr>
<tr>
<td>Diarrhoea, pain, weight loss, clubbing</td>
</tr>
<tr>
<td>Diarrhoea, weight loss, pain, clubbing</td>
</tr>
<tr>
<td>Diarrhoea, weight loss, pain, vomiting, oedema</td>
</tr>
<tr>
<td>Diarrhoea, weight loss, pain, vomiting, clubbing</td>
</tr>
<tr>
<td>Diarrhoea, ankle oedema</td>
</tr>
<tr>
<td>Abdominal discomfort, ankle oedema</td>
</tr>
<tr>
<td>Diarrhoea, pain, growth retardation, clubbing</td>
</tr>
<tr>
<td>Diarrhoea, growth retardation, leg ulceration</td>
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</table>

intestinal biopsies were incubated on a millipore raft in 2 ml RPMI 1640 culture medium (Gibco, New York) with 10% fetal calf serum and 1 μCi each of 14C-labelled leucine, isoleucine, valine, lysine, and arginine (Amersham, UK) for 24 hours at 37°C in 5% CO2: 95% O2. Two mM PMSF (phenyl-methyl-sulphonyl-fluoride) was added to the culture supernate, and particulate material removed by centrifugation. The biopsy was homogenised in 2 ml 50 mM Tris, 100 mM KCL buffer containing triton X100, 0-5% deoxycholate and 2 mM PMSF and cellular debris removed by centrifugation at 90 000G for two hours. Immunoprecipitation of radiolabelled sIgA from the culture supernate and cell lysate was achieved by the addition of 5 μl of purified colostal sIgA and 60 μl of anti-secretory IgA shown to be specific for alpha-heavy chains only. The precipitates containing the in vitro labelled newly synthesised sIgA were washed four times by centrifugation, first with Tris KCL buffer and then with sodium chloride. The immunoprecipitated material was solubilised by boiling under reducing conditions and electrophoresed on SDS 9% polyacrylamide slab gels. 14C-labelled marker proteins (Amersham, UK) were run in parallel with the samples. Following fixation and drying of the gels, autoradiographs were developed after 14 days incubation at -80°C.

serum of each patient was also analysed by molecular sieving on Sepharose AcA34 (LKB) and the fractions electrophoresed on polyacrylamide gradient gels (5-15%). The alpha-chain antigen was identified by an immunoblottinf technique and the molecular size determined from molecular weight markers run concurrently. The IgA content of the fractions was determined by a nephelometric method (Hoechst Nephelometer).

TISSUE CULTURE
Intestinal biopsies were incubated on a millipore raft in 2 ml RPMI 1640 culture medium (Gibco, New York) with 10% fetal calf serum and 1 μCi each of 14C-labelled leucine, isoleucine, valine, lysine, and arginine (Amersham, UK) for 24 hours at 37°C in 5% CO2: 95% O2. Two mM PMSF (phenyl-methyl-sulphonyl-fluoride) was added to the culture supernate, and particulate material removed by centrifugation. The biopsy was homogenised in 2 ml 50 mM Tris, 100 mM KCL buffer containing triton X100, 0-5% deoxycholate and 2 mM PMSF and cellular debris removed by centrifugation at 90 000G for two hours. Immunoprecipitation of radiolabelled sIgA from the culture supernate and cell lysate was achieved by the addition of 5 μl of purified colostal sIgA and 60 μl of anti-secretory IgA shown to be specific for alpha-heavy chains only. The precipitates containing the in vitro labelled newly synthesised sIgA were washed four times by centrifugation, first with Tris KCL buffer and then with sodium chloride. The immunoprecipitated material was solubilised by boiling under reducing conditions and electrophoresed on SDS 9% polyacrylamide slab gels. 14C-labelled marker proteins (Amersham, UK) were run in parallel with the samples. Following fixation and drying of the gels, autoradiographs were developed after 14 days incubation at -80°C.

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Table 2  Histological features and immunoperoxidase evaluation of small bowel biopsies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histological features</th>
<th>Immunoperoxidase evaluation*</th>
<th>Alpha-chains (serum)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td>GW</td>
<td>TVA+LPCI, most atypical</td>
<td>0</td>
<td>trace</td>
</tr>
<tr>
<td>TC</td>
<td>PVA+LPCI, atypical immunoblasts</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Villi normal. Slight increase in plasma cells†</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CC</td>
<td>TVA+PCI</td>
<td>+++</td>
<td>trace</td>
</tr>
<tr>
<td>JK</td>
<td>PVA+LPCI, lymphoma</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>JJ</td>
<td>TVA+PCI</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>SN</td>
<td>PVA+PCI</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

* Scale 0 to ++ (subjective). † At time of study, if different from presentation. TVA = total villous atrophy, LPCI = lymphoplasmacytic cellular infiltration, PVA = partial villous atrophy, PCI = plasmacytic cellular infiltration, FVA = focal villous atrophy.

LYMPHOCYTE CULTURE

Lymphocytes, isolated as previously described, were suspended in RPMI 1640 medium supplemented with 15% AB or autologous serum. After six days incubation with 50 μg of pokeweed mitogen (Gibco, New York) the cells were centrifuged, assayed for cell number and viability, then resuspended in 2 ml of the same medium as for the biopsy cultures containing 14C-labelled amino acids. After a further 24 hours incubation at 37°C, the supernatant fluid and the cells were separated and processed in an identical manner to the culture supernatant and cell lysate from the biopsy cultures.

CELLULAR IMMUNITY

Phytohaemagglutinin (PHA) and concanavalin A (Con A) stimulated lymphocyte transformation was measured by incorporation of 14C-thymidine into peripheral blood lymphocytes. T-cell numbers were determined by their rosetting with uncoated sheep red blood cells with the addition of 2-amino ethylisothiouronium bromide hydrobromide (AET) to stabilise the rosettes. Measurements of T-cells, T-suppressor cells and T-helper cells were done using standard immunofluorescent techniques with commercial monoclonal antisera (OK T series: Ortho Diagnostics, USA) and fluorescent conjugated rabbit anti-mouse IgG antisera (Miles-Yeda, Israel).

RESULTS

BIOPSIES

The abnormalities present on light microscopy at the time of the study reflected those found at presentation (Table 2) with the exception of three cases: patient CC had a completely normal jejunal mucosa eight years after treatment of α-CD with IAL; and the histology of patients TC and CN showed normal villi with little increase in plasma cells in the lamina propria only. These three were asymptomatic at the time of study and only TC was receiving medication (tetracycline). Cases GW, JK, UB and PF, in addition to a dense plasma cell infiltrate, had collections of atypical large lymphoid and plasmacytoid cells. These cells did not take up the immunoperoxidase stains – which were suggestive of α-CD in CC (at presentation only) and TC. Uptake was virtually nil during the active untreated phase of α-CD in patient GW. The immunoperoxidase profile returned to normal after treatment.

SERUM PROTEINS

The serum albumin was low in 10 patients. Four had a raised α2 globulin, three had both raised α2 and β globulins and one had a raised β globulin only. Although TC, CC, HA, JJ and SN had raised IgA (normal 0-64–5.44 g/l) at presentation, only HA (27.3 g/l) and SN (51.0 g/l) had raised levels at the time of study. Free alpha-heavy chains were demonstrable by immunoselection in the serum of patients GW and TC. This method, however, was unable to detect free alpha-chains in the concentrated urine specimens, biopsy homogenates or jejunal juice samples of their, or any of the other patients. The immunoblotting technique confirmed α-CD in GW and TC but was negative in the remainder. Large amounts of IgA polymers, dimers and higher polymers were found in the serum of HA and SN who had PCI with markedly raised serum IgA. This was confirmed by the molecular sieving technique (Fig. 1).
**TISSUE CULTURE**

In those patients with demonstrable free alpha chains in the serum, an abnormal labelled band with a molecular weight of approximately 41,000 was specifically precipitated by anti-sIgA antibody (Figs. 2 and 3). In addition, radioactive labelled secretory piece and the heavy and light chains of reduced normal sIgA with molecular weights of approximately 80,000, 60,000 and 24,000 respectively were seen after polyacrylamide gel electrophoresis. The abnormal alpha-chain band in GW (Fig. 2) was more prominent in the culture supernatant than in the cellular extracts. This band disappeared after treatment with tetracycline and corticosteroids and coincided with the reappearance of demonstrable light chain production. A very broad alpha-chain band was present in tissue culture of TC (Fig. 3). Although this was more prominent in the cellular extract, it was nonetheless also present in the culture supernatant. In the remaining patients no abnormal bands were detected either in the cellular or culture supernatant precipitates, and only the reduced components of normal sIgA were detected.

**LYMPHOCYTE CULTURE**

Analysis of the immune precipitates obtained from the peripheral blood lymphocyte cultures from the patients were all identical and showed only bands corresponding to normal IgA. No abnormal bands were found in lymphocyte cultures in those patients with free alpha-chains in the serum.

**CELLULAR IMMUNITY**

The results are shown in Table 3. In general, lymphocyte responses to transformation were normal or better than normal, except for patient HA. Results obtained with AB and autologous serum supplemented cultures were no different. T-cell...
were correspondingly were the Discussion were rosettes cases. malabsorption Early patient TC alpha-chains in alpha-chains the serum the diagnosis should not be ruled out until a search has been made at the cellular level, it is only recently that a non-secretory alpha-CD variant (by analogy with non-secretory myelomas) has been observed. This has increased speculation that perhaps all cases of IPSID are in fact either secretory or non-secretory forms of alpha-CD.

In the present series only those patients with demonstrable alpha-chains in the serum (GW and TC), showed synthesis of an abnormal alpha-CD protein band in biopsy cultures. In the case of GW, who had a dense cellular infiltrate, this band of precipitation was much more prominent in the culture supernatant than intracellularly. This is in accordance with the previously observed pattern of rapid export of proteins out of the cell and little or no intracellular storage. This band disappeared after treatment, parri passu with normalisation of the serum tests. On the other hand, the alpha-CD protein precipitate of TC, who was partially treated and whose biopsy showed only minimal abnormality, was more dense intracellularly than in the culture supernatant. The explanation for this phenomenon is not clear but may be because of partial postsecretory proteolysis of the deleted fragment of heavy chain. Alternatively, one may postulate that a block in cellular export of the abnormal protein may be one of the early cellular consequences of intervention that is, a reversal of the normal sequence of events resulting from antibiotic therapy.

Alpha-chains could not be identified by a variety of intracellular or extracellular techniques in the remaining eight patients (four with PCI and four with IAL). Although it is possible that some of the methods used were not sufficiently sensitive to

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**Table 3  In vitro tests of cellular immunity**

<table>
<thead>
<tr>
<th>Patient</th>
<th>PHA</th>
<th>Con A</th>
<th>Patient</th>
<th>Control</th>
<th>OKT 3</th>
<th>OKT 4</th>
<th>OKT 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>64</td>
<td>95</td>
<td>49%</td>
<td>62%</td>
<td>63%</td>
<td>45%</td>
<td>38%</td>
</tr>
<tr>
<td>CN</td>
<td>133</td>
<td>130</td>
<td>60%</td>
<td>70%</td>
<td>85%</td>
<td>51%</td>
<td>22%</td>
</tr>
<tr>
<td>JJ</td>
<td>132</td>
<td>70</td>
<td>72%</td>
<td>58%</td>
<td>88%</td>
<td>58%</td>
<td>29%</td>
</tr>
<tr>
<td>SN</td>
<td>167</td>
<td>232</td>
<td>51%</td>
<td>58%</td>
<td>59%</td>
<td>32%</td>
<td>34%</td>
</tr>
</tbody>
</table>

PHA = phytohaemagglutinin, Con A = concanavalin A, OKT 3 = total T-cells (control mean = 62%), OKT 4 = T-Helper cells (control mean = 51%), OKT 8 = T-Suppressor cells (control mean = 31%).
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recognise small amounts of α-CD protein (immuno-
fluorescent studies could not be used for a variety of
reasons), even tissue culture and immuno-
precipitation techniques failed to identify any new
cases of α-CD.

The failure of the immunoperoxidase stains to be
taken up by many of the atypical cells would account
for the inability of this method to diagnose α-CD in
patient GW – which was confirmed by other
methods. The active alpha-chain secreting cells were
probably ‘crowded out’ by the mass of non-alpha-
chain producing atypical cells, and may possibly
have been identified by another technique. The
PAP method easily confirmed α-CD in TC, how-
ever, whose biopsy was only slightly abnormal.
Although lymphoma cells may derive from the same
close as the α-CD protein secreting cells,25 lack of
secretory differentiation of the lymphoma cell mass
would account for the absence of detectable α-CD
protein in patients with IAL. Abnormal alpha-
chains could not be detected in in vitro cultures of
precursor peripheral blood lymphocytes in the cases
with α-CD or any of the other patients.

It has been suggested that patients with IPSID
may have a defect in cell mediated immunity.1 A
possible deficiency of suppressor T-cells may allow
uncontrolled proliferation of plasma cells in re-
Sponse to an antigenic stimulus. By the in vitro
techniques described there appeared to be no
significant abnormality in cellular immunity in the
four PCI patients. Two patients, however, did show
a relative decrease in T-helper cells and corres-
ponding increase in T-suppressor cells, the signifi-
cance of which is uncertain.

Patients HA and SN share many features with a
previously described unclassified case.26 Both are
young and have raised polymerised serum IgA with
raised serum viscosities. Predominance of polymer-
ised serum IgA is a feature of IgA myeloma, which
is known to predispose to a hyperviscosity syn-
drome. No evidence of myeloma could be found in
any of the cases. Patient HA developed bilateral
axillary vein thrombosis in 1982. Thus the ‘benign’
cellular infiltrate of the bowel may have serious
repercussions and consideration must be given to
the consequences of increased serum viscosities in
this group of patients, particularly during episodes
of diarrhoea to which they are prone.

The patients with PCI and the clinical criteria of
IPSID, but without serological evidence of α-CD or
lymphoma, are an interesting poorly defined sub-
group.9 As a group these patients have caused
confusion as both inflammatory and neoplastic
processes have been incriminated.20 27 28 Despite
continuing exposure over a prolonged period to
whatever environmental stimuli may be operative,
we have not observed any case evolve to overt α-CD
or lymphoma. We have also been unable to show a
basic deficit in cell mediated immunity or non-
secretory alpha-chain production in these patients.

We wish to thank our many colleagues who assisted
in the investigation and management of the patients
described. Support from the Medical Research
Council of South Africa is gratefully acknowledged.

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