Immunoglobulin gene rearrangement in immunoproliferative small intestinal disease (IPSID)

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SUMMARY Analysis of DNA from the mucosal tissue of three patients with immunoproliferative small intestinal disease (IPSID) and α chain disease, two of whom had early stage disease responsive to antibiotics, showed monoclonal heavy and light chain gene rearrangements in all cases. These findings suggest that IPSID is neoplastic even in its early stages, but that the neoplastic cells respond to normal stimuli. Monoclonal lymphoid populations could not be detected in circulating lymphocytes from these patients, which raises the possibility that the circulatory pathways of lymphocytes derived from human gut associated lymphoid tissue may not necessarily parallel those in experimental animals.

Immunoproliferative small intestinal disease (IPSID) occurs predominantly, but not exclusively, in the Middle East, and is characterised by diarrhoea, weight loss, abdominal pain and finger clubbing.1 2 The pathological basis of these features is a heavy plasmacytic or lymphoplasmacytic infiltrate of the upper small intestinal mucosa and related mesenteric lymph nodes. In most, if not all, cases these plasma cells synthesise, without necessarily secreting,3 an abnormal α1 immunoglobulin heavy chain (α chain disease). Malignant intestinal lymphoma (Mediterranean lymphoma) often arises in the setting of IPSID and opinions are divided as to whether IPSID is a malignant proliferation de novo or whether the onset of Mediterranean lymphoma represents a complication of a benign disorder.1 2 4 The latter view is supported by the evident response of some cases of uncomplicated IPSID to broad spectrum antibiotics1 2 4 5 while cases of Mediterranean lymphoma require more radical treatment. Decisions regarding treatment are based on the interpretation of the histology of intestinal biopsy specimens.4 The histopathological differential diagnosis between uncomplicated IPSID and Mediterranean lymphoma can, however, be extremely difficult and is subject to sampling error.

Central to the argument on the nature of the lymphoproliferation in IPSID, and hence the optimum choice of treatment, is the clonality of the mucosal plasma cell infiltrate in the early stages of the disease. The absence of light chains and the loss of the variable region of the abnormal α1 heavy chain precludes the use of the two most common criteria of monoclonality: light chain restriction and monoclonal idiotype expression. Although cases showing light chain restriction have been described,6 7 these were in established cases of Mediterranean lymphoma rather than in examples of early IPSID. Accordingly, we analysed the DNA from three cases of IPSID for evidence of monoclonal rearrangements of both heavy and light chain immunoglobulin genes. Two cases were examples of early IPSID that had responded to conservative treatment and one was of established Mediterranean lymphoma. All three patients were from the Cape region of South Africa, where IPSID is a well recognised disease.

Patients and methods

CASE 1
A 26 year old woman complained of diarrhoea and abdominal pain associated with a weight loss of 6 kg. Apart from emaciation, there were no abnormal physical findings. Gastroduodenoscopy showed slight nodularity of the first part of the duodenum. The only clinically important biochemical finding was the presence of serum α chains. The duodenal biopsy specimen was consistent with a diagnosis of IPSID and the

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patient underwent a staging laparotomy with full thickness jejunal and mesenteric lymph node biopsy, at which time fresh tissue was collected. Treatment consisted initially of metronizadol to eradicate *Giardia* followed by long term tetracycline 1 g twice a day. Six months after diagnosis the patient remained well, having regained her original weight. Serum $\alpha$ chains were undetectable. Fourteen months after diagnosis, when peripheral blood was taken, she remained well and was 30 weeks pregnant at the time of writing.

**CASE 2**

A 20 year old man gave a four year history of diarrhea and a weight loss of 18 kg. Examination showed that he was physically wasted with no other clinically important findings. Gastroduodenoscopy yielded normal results as did biochemical investigations, except for the presence of serum $\alpha$ chain. Laparotomy with full thickness jejunal and mesenteric lymph node biopsy was carried out and fresh tissue retained. Peripheral blood was collected simultaneously. The patient started on tetracycline 1 g twice a day and parenteral nutrition—with pronounced clinical improvement. A follow up jejunal biopsy at four months was unchanged.

**CASE 3**

A 15 year old boy gave a three month history of abdominal pain, weight loss, and diarrhea. Examination showed general wasting and clubbing of his nails. Gastroduodenoscopy showed nodular infiltration of the duodenum; serum $\alpha$ chain was present. Laparotomy showed thickening of the entire small intestine with enlarged mesenteric lymph nodes, and full thickness jejunal and mesenteric lymph node biopsy specimens were taken and submitted fresh. Prednisone 40 mg daily, tetracycline 500 mg twice daily, and parenteral nutrition were started. At follow up 15 months after admission the patient remained asymptomatic and $\alpha$ chain was still detectable in his serum. Peripheral blood was collected at this time. Follow up biopsies were refused by the patient who then received total abdominal irradiation with good clinical response.

Standard formalin fixed, paraffin embedded sections from the full thickness jejunal and mesenteric lymph node biopsy specimens were prepared and stained with haematoxylin and eosin. Immunohistochemistry was performed on these and on cryostat sections prepared from fresh tissue, snap frozen in liquid nitrogen. Sections were stained with antibodies to all immunoglobulin heavy and light chain and selected B cell and T cell antigens.

Frozen sections were prepared from tissue snap frozen in liquid nitrogen to check that the tissue being submitted for DNA analysis was representative. After collection in edetic acid, lymphocytes from peripheral blood from all three patients were separated on a Ficoll hypaque gradient, harvested, and snap frozen in liquid nitrogen. High molecular weight DNA was extracted by standard methods from the biopsy specimens and peripheral blood lymphocytes. After digestion with HindIII, EcoRI, BamHI, and BgIII restriction enzymes the fragments were separated according to size on an agarose gel by electrophoresis and Southern blots prepared using Gene Screen Plus filters (DuPont). Table 1 details the recombinant DNA probes used and their sources. They were radio-labelled with $^{32}P$-dCTP by the random hexanucleotide method and hybridised to the filters under conditions recommended by the manufacturer. After stringent washing the filters were exposed to pre-fogged x-ray film.

**Results**

The histological appearances (table 2) of the biopsy specimens in cases 1 and 2 showed a dense plasma cell infiltrate confined by the muscularis mucosae. This was focal in case 1, whose biopsy specimen also showed lymphoepithelial lesions, and diffuse in case 2. The lymph node of case 1 showed reactive hyperplasia while that of case 2 showed infiltration with mature plasma cells. The small intestinal biopsy specimen of case 3 showed a nodular mucosal lypho-plasmacytic infiltrate which breached the muscularis mucosae. Lymphoepithelial lesions were present; lymph node architecture was effaced by a similar infiltrate.

Immunohistochemistry of paraffin sections showed cytoplasmic Ig of the $\alpha$, subclass in the plasma cells of all patients. No light chain was shown in cases 1 and 3, but $\kappa$ light chain restriction was present in case 2. Using frozen sections the mucosal lymphocytes in case 1 were Slg negative while in case 3 Slg$\kappa$, without light chain was demonstrable. Mucosal lymphocytes were virtually absent in case 2.

DNA analysis (table 3, figure) using the Jh probe showed the presence of a clone of B cells in all three biopsy specimens. Rearrangements were detected with four different restriction enzymes. In samples from cases 1 and 3 only one rearranged band was detected. Both alleles, however, exhibited a non-germline configuration in case 2. Only germline frag-

<table>
<thead>
<tr>
<th>Table 1 Recombinant DNA probes used</th>
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<tbody>
<tr>
<td><strong>Probe</strong></td>
</tr>
<tr>
<td><strong>Code name</strong></td>
</tr>
<tr>
<td><strong>Reference</strong></td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Heavy chain joining region (Jh)</td>
</tr>
<tr>
<td>C76R51A</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>$\kappa$ light chain constant region (C$\kappa$)</td>
</tr>
<tr>
<td>pUCR17</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>$\lambda$ light chain constant region (C$\lambda$)</td>
</tr>
<tr>
<td>pUC15</td>
</tr>
<tr>
<td>14</td>
</tr>
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</table>
ments hybridising to JH were observed in DNA extracted from the three blood samples.

The probing of BglIII and BamH1 digests of DNA from cases 1 and 3 with the Cκ probe yielded germline fragments. Analysis of the Cl locus in the DNA extracted from all three biopsy specimens indicated the presence of hybridising fragments which did not resemble any published germline patterns. Probing DNA extracted from peripheral blood lymphocytes from the patients with Cl, however, showed a pattern of hybridising fragments which differed from that seen in the DNA of the biopsy specimens. We detected a similar pattern in several control samples (unpublished results) and it resembles a published germline Cl configuration. Rearrangement of both the Cκ alleles was detected in the DNA from the biopsy specimens from case 2, but not from the peripheral blood lymphocytes.

Discussion

The presence of a clone of B cells in each biopsy specimen was shown by the non-germline fragments which hybridise to the JH probe in DNA purified from these samples. The use of four different restriction enzymes excludes the possibility that polymorphisms at this locus are responsible for these additional bands. Rearrangement of the immunoglobulin heavy chain (IgH) locus occurred at one allele in cases 1 and 3, while this occurred at both alleles in case 2. In this case three of the four restriction enzymes showed two non-germline fragments while three EcoRI fragments hybridised to the probe in addition to the germline fragment. It would seem likely from the data concerning the three restriction enzymes that rearrangement had occurred in two alleles but that one of the rearrangements had introduced an additional EcoRI site into the region hybridising to the JH locus. This may have occurred by the deletion of DNA 3' to the Cα locus during somatic recombination (Rabbitts TH, personal communication). Cloning and sequencing the rearranged fragments would elucidate the somatic recombination mechanisms. Similar sized EcoRI fragments have been observed in genetic analysis of acute undifferentiated leukaemia cells.

Probing of the BamH1 and BglIII digests showed that only germline bands hybridising to Cκ were present in DNA from cases 1 and 3. Both Cκ alleles had undergone rearrangement in the biopsy specimens from case 2. Analysis of DNA from non-T/non-B leukaemias led Korsmeyer et al. to postulate a developmental hierarchy in which IgH gene rearrangement precedes that of κ, which in turn precedes that of λ. As our data do not suggest that Cκ rearrangement occurred in cases 1 and 3 despite the Cl rearrangement, three possible hypotheses can be put forward. The Cκ genes in the clone may have been deleted, but this was masked by the presence of non-clonal cells which still retained the Cκ germline configuration. Alternatively, the Cκ genes in the clone may have undergone rearrangement in such a manner to recreate the germline fragments, but this is unlikely as both enzymes indicate rearrangement. The third possibility is that this hierarchy does not apply in IPSID.

Interpretation of the data obtained with the Cl probe was complicated by the nature of the locus: it is highly polymorphic and composed of between six and nine genes per haploid genome. Comparison of the patterns obtained from the DNA purified from the biopsy specimens with that extracted from the peripheral blood lymphocytes suggested that rearrangement of this complex locus had occurred in biopsy specimens from all three patients. If no rearrangement had occurred it could be predicted that the patterns obtained from the tissue would be identical with that in peripheral blood leucocytes which showed no evidence of clonal rearrangements.

The demonstration of rearrangement in the λ light chain loci in cases 1 and 3, despite the lack of immunohistochemical evidence for synthesis of λ light chain, can be interpreted in several ways. The first possibility is that λ chains were present at the cell surface but only at levels not detectable by immunohistochemistry. The second is that both light and heavy Ig genes were correctly transcribed and translated, but that the assembly of the complete molecule or its transport to the membrane was impaired. Alternatively, the Cl rearrangement may have generated a gene with incorrect control signals such as initiation, splicing, or termination, which could not be correctly

Table 2  Histological appearances of biopsy specimens

<table>
<thead>
<tr>
<th>Histology/immunocytochemistry</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoplasmacytic infiltrate</td>
<td>Mucosal</td>
<td>Mucosal</td>
<td>Mucosal and submucosal</td>
</tr>
<tr>
<td>Lymphoid nodules</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Hyperplasia</td>
<td>Plasma cells</td>
<td>Infiltrated</td>
</tr>
<tr>
<td>Cytoplasmic a, heavy chain</td>
<td>Absent</td>
<td>Plasma cells</td>
<td>Plasma cells</td>
</tr>
<tr>
<td>Surface a, heavy chain</td>
<td>Absent</td>
<td>Absent</td>
<td>Present (lymphocytes)</td>
</tr>
<tr>
<td>Light chain</td>
<td></td>
<td></td>
<td>Absent</td>
</tr>
</tbody>
</table>
table 3 dna analysis using jh probe

<table>
<thead>
<tr>
<th>case no</th>
<th>hind ii g = 9.5 kb</th>
<th>jh*</th>
<th>eco ri g = 19.0 kb</th>
<th>bam hi g = 18.0 kb</th>
<th>bgl iii g = 3.8 kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 biopsy</td>
<td>germ line + rearranged (7)</td>
<td>germ line + rearranged (2-6)</td>
<td>germ line + rearranged (30)</td>
<td>germ line + rearranged (32)</td>
<td></td>
</tr>
<tr>
<td>peripheral lymphocytes</td>
<td>germ line</td>
<td>germ line + rearranged (7-2, 5-6)</td>
<td>germ line + rearranged (5, 2-7, 2-5)</td>
<td>germ line + rearranged (7, 7-7)</td>
<td></td>
</tr>
<tr>
<td>2 biopsy</td>
<td>germ line + rearranged (13)</td>
<td>germ line + rearranged (12)</td>
<td>germ line + rearranged (8-7)</td>
<td>germ line + rearranged (14)</td>
<td></td>
</tr>
<tr>
<td>peripheral lymphocytes</td>
<td>germ line</td>
<td>germ line</td>
<td>germ line</td>
<td>not done</td>
<td></td>
</tr>
</tbody>
</table>

*represent sizes of rearranged bands in kilobases. for cl band sizes of all hybridising fragments are detailed. fragment sizes which are outside the range of the molecular weight markers used are indicated by 30+.

transcribed or translated. the analysis of light chain genes in case 2, whose tumour showed k light chain restriction, showed the unusual, but not unprecedented, evidence of both k and l rearrangements.

histopathologists recognise three stages of ipsid. in the first stage (a) a benign appearing plasma cell or lymphoplasmacytic infiltrate is confined to the mucosa and mesenteric lymph nodes. in stage b cytologically neoplastic cells begin to appear in this infiltrate which extends into the submucosa and beyond, while in stage c there is frank recognisable lymphoma. patients in stage a may respond to antibiotics alone (as is well shown by case 1 in this report, and, to a lesser extent, by case 2). the pathology in case 3 is that of stage b and his failure to respond to conservative treatment was expected. in view of the response of some patients to antibiotics alone it has been suggested that stage a is a benign non-lymphomatous disorder. the implication is that the plasma cell proliferation is polyclonal at this stage, with a single clone emerging as the disease progresses. until the advent of dna analysis of immunoglobulin gene rearrangements, monoclonal lymphoproliferation could only be shown by showing light chain restriction or, using anti-idiotype serum, a single idiotype in the synthesised immunoglobulin. characteristically, light chains are only rarely synthesised in ipsid; nevertheless, the finding of light chain restriction in the plasma cells of some cases and in case 2 in this report, strongly supports a monoclonal proliferation. the use of anti-idiotype sera is vitiated in ipsid as the immunoglobulin domain responsible for the synthesis of idiotype—namely, the variable region—is characteristically absent from the

examples of filter hybridisations of dna extracted from cases 1, 2, and 3 probed with jh, ck, and cl. rearranged fragments are indicated by r, germ line by g. molecular weight markers and dna fragment sizes are all in kilobases. lanes labelled b illustrate dna extracted from biopsy specimens. those labelled pb are from peripheral blood lymphocytes. restriction enzymes used are given beneath each example.
Ig gene rearrangement in IPSID

\[ Ig \] gene

rearrangement

in IPSID

\[ CK^* \]

\[ CA \]

\[ BamH1 G = 120 \text{ Kb} \]

\[ BglII G = 130 \text{ Kb} \]

\[ EcoRI \]

\[ HindIII \]

Germline

Germline

Germline + rearranged
(10, 1, 7)

Germline

Germline

Germline

Germline + rearranged
(15, 8, 6, 7)

Germline

Germline

Germline + rearranged
(10, 9, 5, 3)

Germline + rearranged
(16, 10, 9, 5, 3)

Not done

Germline + rearranged
(15, 8, 6)

Germline

Germline + rearranged
(21, 10, 9, 7, 5)

Germline

Germline + rearranged
(21, 18, 5, 8, 6, 7-3)

Germline

Germline + rearranged
(21, 8, 6)

Germline

Germline

Germline

Germline + rearranged
(21, 18, 5, 8, 6, 7-3)

Germline

Germline + rearranged
(21, 8, 6)

Germline

Germline

Germline

Germline + rearranged
(21, 18, 5, 8, 6, 7-3)

Germline

Germline + rearranged
(21, 8, 6)

Germline

Germline

Germline

Germline + rearranged
(21, 18, 5, 8, 6, 7-3)

Germline

Germline + rearranged
(21, 8, 6)

Germline

Germline

Germline

Germline + rearranged
(21, 18, 5, 8, 6, 7-3)

Germline

Germline + rearranged
(21, 8, 6)

\[ a_1 \] heavy chain in this disease. The demonstration of clonal rearrangements of immunoglobulin genes in our three patients, two of whom were in stage A, is, we believe, clear evidence that the lymphoproliferation in IPSID is monoclonal from the start.

Given that clonality is the cardinal feature of neoplasia, one must conclude that IPSID is lymphomatous in all its stages. Our findings are comparable with those of Weiss et al.\(^ {22} \) who recently carried out DNA analysis on cases of lymphomatoid papulosis of the skin, an apparently benign disorder which progresses to T cell lymphoma in 20% of cases. Using DNA analysis Weiss et al found evidence of T cell monoclonality in the benign stage of the disease and suggested that at this stage the clonal population and host response are in balance. This could well be the case in stage A of IPSID where the neoplastic clone seems to be responding normally to a stimulus of lumenal bacterial overgrowth and involuting when this stimulus is removed by broad spectrum antibiotics. Claims of “cure” using antibiotics alone should be treated with caution as IPSID can have a long natural history before lymphomatous masses become evident, and relapses after an initial response to antibiotics are common.\(^ {4} \) In view of our findings should all cases of IPSID receive chemotherapy or radiotherapy regardless of stage? Unless one can be certain that this form of treatment will destroy the malignant clone, it is probably wise to withhold it until such time as antibiotics fail to control the disease. Newer forms of treatment for lymphoid malignancy, possibly immunologically based, however, must surely emerge and the knowledge that IPSID is a neoplasm de novo is important to keep in mind in
this respect. Furthermore, when follow up is likely to be difficult it may be prudent to treat patients with a lymphoma regimen regardless of the pathological stage.

Studies on animals have shown that mucosal plasma cells are derived from Peyers patch lymphocytes indirectly via the mucosal lymphatics and the circulation. We therefore might have expected to detect a monoclonal population of cells in the circulation in our cases, especially in cases 2 and 3 with active disease. Such clones have indeed been detected by Southern blotting in cases of low grade nodal non-Hodgkin’s lymphoma, including cases in remission. In view of our findings and as the evidence for the circulatory phase of gut associated lymphoid tissue is entirely animal derived, its presence in man should perhaps be questioned.

The epidemiology of IPSID together with reports of specific HLA association points to a genetic predisposition in this disease, which when combined with one or more environmental factors leads to abnormal IgA synthesis and lymphoma. The use of further molecular techniques together with cytogenetic studies of patients and their families is indicated to elucidate the molecular biology of IPSID. The findings of such a study would be relevant not only to IPSID, but to the pathogenesis of B cell lymphoma as a whole.

Support from the Cancer Research Campaign is gratefully acknowledged.

References

13. Bentley DL, Rabbitts TH. Evolution of immunoglobulin V genes: evidence that recently duplicated human Vk sequences have
Ig gene rearrangement in IPSID

14 Rabbitts TH, Forster A, Matthews JG. The breakpoint of the Philadelphia chromosome 22 in chronic myeloid leukaemia is distal to the immunoglobulin \( \lambda \) light chain constant region genes. Mol Biol Med 1983;1:11–9.
26 Requests for reprints to: Professor PG Isaacson, Department of Histopathology, School of Medicine, University College London, University Street, London WC1E 6JJ, England.