Author's response to reviews

Title: Hyper-IgG4 disease: Report and characterisation of a new disease.

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Author’s response to reviews: see over
Dear Editors

Thank you for sending us the reviewers’ helpful comments.

We have now addressed their comments and indicated in our reply (below) the changes that have been made to the text.

In reply to your own editorial comments:

1. Ethics.

This was a retrospective review of case notes and an investigation of archival biopsy material. We have now inserted the following sentence into the Methods (page 5):-

“Only archival paraffin-embedded material was used for this study. All the biopsies were taken for diagnostic reasons with the informed written consent of patients”.

2. We have also added to the Acknowledgements:

“Written consent was obtained from the patient for publication of the Case history.”

We will forward this on to you.

We hope that you will now find all the necessary changes and additions satisfactory.

Yours Sincerely

Guy Neild and Manuel Rodriguez-Justo (on behalf of the co-authors)
Reviewer: 1 Terumi Kamisawa  (date 5 June 2006)

Major compulsory revision
1. Response of RPF to steroid therapy.

We have already emphasized in the paper that during the active period of the disease, while there is evidence of an acute phase response, there is typically a rapid clinical response to steroids. We have now added a paragraph concerning treatment of RPF (see below) in the Discussion at the end of the first introductory section on RPF (page 10).

“Although there are no randomised studies of treatment, patients who present during the acute inflammatory phase respond quickly to gluco-corticosteroids. Patients with late presentation and dense fibrosis will not respond to medical treatment and surgery is often necessary [11].”

2. Do authors think that all idiopathic RPF are involved in this new disease?

We have now discussed this important point in a short new section called Summary at the end of the Discussion of RPF (see page 13).

“Although all our archival cases of idiopathic RPF have shown evidence of IgG4 involvement it remains to be seen whether IgG4 plays any role in other types of RPF such as peri-aortitis, drug-induced and asbestos-related RPF. We certainly found it (in retroperitoneal tissue) in one of our cases who developed pleural calcification.”

We have also added a sentence in the Asbestos section (p 11) stating that one of our cases (Case 5) developed the typical pleural calcification attributed to Asbestos (See below).

We have also seen pleural calcification in 3 of our patients with RPF in whom it was not present at the presentation of the illness. “One of those cases is included in our archival review (Case 5, Table 4)“.

Minor essential revision
1. IgG4-related systemic disease.

We acknowledge that others have already reported systemic disease and have added in a further paragraph to the Background (see page 4).

Kamisawa and his colleagues have also published several reports emphasising the systemic nature of this process. Patients with autoimmune pancreatitis may also have involvement of lymph nodes and salivary glands as well as local tissues [7, 8] in a pattern suggestive of multifocal fibrosclerosis [9,10].

2. Too many histopathological figures

We have now deleted 50% of the figures.
Specific Comments

Comment 1:

We had only described the pathological findings in RPF as this was the main purpose of the study. We have now added a short paragraph (page 8) to give details of the other biopsy specimens reported in Table 4 (see below).

“We also examined biopsy specimens from liver tissue (2 patients), large bowel (1) and a renal biopsy (1). In all of them we found mild fibrosis and mild increase in a lymphoplasmacytic inflammatory cell infiltrate, although IgG4+ve plasma cells were not seen in one liver and the colon biopsy (see table 4).”

As we have already stated in the Methods (see page 5):

“As positive controls we also refer to tissue obtained from pancreas, salivary gland and liver from patients seen in our gastroenterological department who had sclerosing pancreatitis and chronic inflammation associated with IgG4-expressing plasma cells, details of which have been reported[3,4].”

Figure 4 includes histological data from these reports [3,4] (of a patient with autoimmune pancreatitis type features in which there was also salivary gland involvement and in this case the number of IgG+ve plasma cells was again significantly increased). We now make this clearer in the legend to figure 4.

Figure 4: Histopathology – IgG4 immunostaining: cases vs controls.
Legend: vertical axis (0-120) shows number of IgG4+ve plasma cells/high power field). “See Methods for details. Histological data from salivary gland is positive control from data previously reported [3,4].”

Comment 2:

The details of the biopsy specimens available were listed in the first paragraph of the Results (Pathology of IgG4 disease) – page 8. In addition there were 2 further samples of retroperitoneal tissue taken from 2 patients after steroid therapy. The details of these second biopsies are not shown in Table 4 but are reported already in the text. We have now amended these details so they are less ambiguous (see below.)

Pathology of IgG4 disease

“We have reviewed the histology and examined sections for evidence of IgG4-expressing plasma cells of 16 biopsies from 12 patients with a clinical diagnosis of idiopathic RPF seen at our hospital in the previous 10 years. Biopsies were from retroperitoneal tissue (9 samples), liver (2) and one each from kidney, colon, and omentum. In addition there were 2 further samples of retroperitoneal tissue taken from 2 patients after steroid therapy” (Page 8).

Also to make this all clearer we have added a sentence in the Methods section as well.

“We report on the histological features of 16 biopsy specimens from 12 patients seen at our Hospital with a primary diagnosis of RPF in…. ” (Page 4).
Comment 3:

In our own experience with the 12 cases of RPF reviewed in this paper and 11 patients with Auto-immune Pancreatitis (AIP) (11 with pancreas biopsies, 7-liver biopsy, 2 with upper GI biopsy, 1 with bone marrow biopsy), we have found that in patients with both AIP and “RPF-related Hyper-IgG4 disease” the percentage of plasma cells bearing IgG4 is >80% of the total number of plasma cells. For instance in liver biopsies from patients with AIP where we did not find many plasma cells, more than 80% of these plasma cells showed IgG4+ve staining.

In our own negative controls we have found <3 IgG4+ve plasma cells/HPF, and the percentage of plasma cells expressing IgG4 in negative controls to be 5% or less.

At the end of our comments to Reviewers we have added an Abstract which was recently presented at the Annual Meeting of the Pancreatic Society of Great Britain and Ireland and the Digestive Disease Week in Los Angeles), and we now cite this paper also in the Background (as new reference 4).

Comment 4:

We have addressed the issue of AIP / hyper-IgG4 disease as a systemic disease in our response to Reviewer 1 (see Minor essential revision; 1. IgG4-related systemic disease.)

Comment 5:

In response to the comments about the unnecessary differential diagnosis of i) secondary RPF and ii) FUO we have omitted Tables 5 and 6.
IgG4 immunostaining in autoimmune pancreatitis and in extra-pancreatic disease
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DDW, Los Angeles 2006, Poster #1225

Aims:
Patients with autoimmune pancreatitis (AIP) usually present with a pancreatic mass, jaundice, and pancreatic and bile duct stricturing. Recognition of this entity is vital to avoid unnecessary resection of suspected malignancy. Originally thought to be restricted to Japan, we have recently reported a series of AIP patients from the UK, with a high frequency of extra-involvement. Definitive diagnosis is difficult, relying on a combination of clinical, radiological and biochemical features, and raised serum IgG4. The aim of this study was to investigate the utility of IgG4 immunostaining in the diagnosis of autoimmune pancreatitis and associated extra-pancreatic disease in biopsy specimens.

Methods:
All patients seen in our centre from 2004 to 2005, in whom the diagnosis of AIP was made on the basis of the typical characteristics, were studied. Biopsy specimens from patients and controls were immunostained with human monoclonal IgG4 antibody. IgG4 positive plasma cells were assessed in 10 high power fields (HPF). Correlation was made with serum IgG4 levels.

Results:
IgG4 positive plasma cell numbers were consistently elevated in pancreatic and extra-pancreatic biopsy tissues even in cases with normal serum IgG4. The majority of AIP patients had >10 IgG4 positive plasma cells per HPF. Control tissues showed <1 IgG4 positive plasma cells per HPF. The percentage of total plasma cells which expressed IgG4 was >80% in AIP patients compared with <10% in controls.

Conclusions:
An IgG4 positive plasma cell infiltrate appears to be specific to AIP when compared with other inflammatory disorders and immunostaining may be of use in the routine investigation of intra- and extra-pancreatic disease in clinically suspected autoimmune pancreatitis.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Serum IgG4 N&lt;1.5g/l</th>
<th>Biopsies from clinically involved organs</th>
<th>Numbers if IgG4 positive plasma cells per High Power Field: Patient samples</th>
<th>Numbers of IgG4 positive plasma cells per High Power Field: Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Raised</td>
<td>Liver</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Raised</td>
<td>Kidney, Pancreas, Liver</td>
<td>73, 20, 21</td>
<td>0, 2, 1</td>
</tr>
<tr>
<td>3</td>
<td>Raised</td>
<td>Bone Marrow, Submandibular gland, duodenum</td>
<td>25, 106, 51</td>
<td>0, 2, 0</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>Liver, Pancreas, Gallbladder</td>
<td>8*, 22, 61</td>
<td>1, 2, 2</td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>Pancreas</td>
<td>2***</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>Liver</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>N/A</td>
<td>Liver</td>
<td>8, 20</td>
<td>1, 1</td>
</tr>
<tr>
<td>8</td>
<td>Normal</td>
<td>Liver</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Raised</td>
<td>Liver</td>
<td>84</td>
<td>1</td>
</tr>
</tbody>
</table>

*insufficient portal tract tissue **Control tissues (10 per organ system): Liver (chronic hepatitis C), Pancreas (chronic alcoholic pancreatitis), Gallbladder (chronic cholecystitis), Kidney (tubulointerstitial nephritis), Salivary gland (chronic sialadenitis), Duodenum (chronic duodenitis), Bone marrow (normal bone marrow) ***Severe fibrosis