Deposits of terminal complement complex (TCC) in muscularis mucosae and submucosal vessels in ulcerative colitis and Crohn’s disease of the colon

TS HALSTENSEN, TE MOLLNES, O FAUSA AND P BRANDTZAEG

From the Laboratory for Immunohistochemistry and Immunopathology (LIHPAT), Institute of Pathology, Institute of Immunology and Rheumatology, and Section of Gastroenterology, Medical Department A, University of Oslo, The National Hospital, Rikshospitalet, Oslo, Norway

SUMMARY Extensively washed, ethanol fixed and paraffin embedded colonic specimens from 15 patients with ulcerative colitis (UC) and nine patients with Crohn’s disease (CD) of the colon, ileal specimens from six patients with CD of the ileum, and histologically normal control specimens obtained from 10 patients operated for colonic carcinoma, were examined by immunohistochemistry with a monoclonal antibody specific for a neoepitope in the C9 part of the terminal complement complex (TCC). The submucosal blood vessels in inflammatory bowel disease (IBD) showed significantly more TCC positivity than the controls, and vascular TCC deposition was statistically related (p<0.001) to degree of inflammation. Five of the six ileal CD specimens contained likewise vascular TCC deposits. In addition, five UC specimens and one colonic CD specimen contained TCC-positive fibrils in the muscularis mucosae or submucosa. There was no significant difference in vascular TCC deposits between UC and CD. The results suggested that terminal complement activation takes place in the intestinal lesions of IBD.

The aetiology of inflammatory bowel disease (IBD) is unknown. Several possibilities have been suggested, including infectious agents, autoimmunity and local immune complex deposition.1,2 Despite the lack of definite evidence, it is generally believed that immunological mechanisms contribute to the pathogenesis of ulcerative colitis (UC) and Crohn’s disease (CD). Immunohistochemical studies of diseased mucosa have revealed a marked local over production of IgG in IBD3 with a significantly higher IgG1-to-IgG2 proportion in UC than in CD.2 To some extent the locally produced IgG shows specificity for intestinal microorganisms.5,7 Moreover, local IgG has been found to react with an intestinal epithelial antigen in UC but not in CD.8

As IgG can activate complement, the striking local IgG response seen in IBD is of considerable pathogenetic interest. In 1974 Ballard and Shiner9 proposed that immune complexes formed at the epithelial basement membrane might activate complement and attract polymorphonuclear cells; the release of proteolytic enzymes from these cells could then lead to epithelial destruction.

Immune complexes induce C3 activation either by the classical or alternative pathway. C3b participates in the C5 convertase which initiates the terminal pathway when C5 is cleaved. The terminal complement complex (TCC) is formed when C5b reacts with C6, C7, C8, and C9.

On biological membranes TCC appears in the form of the cytolytic, pore forming C5b-9(m).10 In a fluid phase, however, S-protein (vitronectin) binds to C5b-7 and the non-cytolytic soluble form of TCC (SC5b-9) is formed.10 Both C5b-9(m) and SC5b-9 express TCC neoepitopes which are absent from the native components. The purpose of this study was to examine lesions of IBD for the expression of TCC neoepitope suggestive of local complement activation.

Methods

TISUE SPECIMENS

Colonic tissue from inflamed lesions were obtained immediately after surgical resection in 15 patients...
with UC (average age, 33 years; range, 17–50) and
nine with CD of the colon (average age, 30 years;
range, 15–64, Table 1). In addition, we collected ileal
samples from six patients with CD of the ileum
(average age, 31 years; range, 18–43). The diagnoses
were based on clinical and pathological criteria.
Control material was obtained from histologically
normal mucosa of 10 patients (average age, 71 years;
range, 51–80) undergoing colonic resection because
of carcinoma. These specimens were taken well away
from the tumour.

All specimens were collected in ice cold isotonic
saline and brought to the laboratory within one hour.
Thin tissue slices were washed for 48 h at 4°C in
0·01 M phosphate buffer (pH 7·5) containing 0·15 M
NaCl (PBS) to remove diffusible proteins. There-
after the tissue was fixed for 18 h in cold 96% ethanol,
dehydrated in absolute ethanol, cleared in xylene and
embedded in paraffin for three to four hours at
56°C.12 The paraffin blocks were stored at 4°C until
used.

**Microscopy and photography**

Immunofluorescence features were observed in a
Leitz Orthoplan microscope equipped with an XBO
150 W lamp. The results were recorded on Agfa 1000
ASA daylight film.

**Immunohistochemical evaluation**

The immunofluorescence examination was per-
formed blindly twice by the same investigator. Each
specimen was given a semi-quantitative score ranging
from quite faint fluorescence in a few submucosal
vessels (+) to intense staining in many vessels through
the submucosa (+++). One tissue section from each
immunohistochemical series was stained with
haematoxylin and cosin and was examined blindly.
**TCC in inflammatory bowel disease**

Each specimen was given a semiquantitative inflammatory score from negative (0) to intense (+++) on the basis of the cellular infiltrate density.

**CONTROLS**

Incubation with murine control ascites at a dilution comparable to that used for the MoAb (1:4000), did not produce detectable staining. Occasional weak autofluorescence was seen, however, in the internal elastic membrane of some vessels.

**STATISTICAL ANALYSIS**

Comparisons between specimen categories were based on the Mann-Whitney non-parametric two-tailed test. Correlation analyses were performed by the Kendall's t test.

**Results**

Intense staining for TCC in submucosal blood vessels was found by indirect immunofluorescence in 14 of 15 UC specimens and in eight of nine colonic CD specimens (Fig. 1 and Table 1). Five of six ileal CD specimens were likewise positive for TCC (Table 2). By contrast, only one of 10 histologically normal colonic control specimens showed positive vascular staining for TCC (p<0.01) (Table 3). Re-examination with the sensitive APAAP method, however, revealed some deposits in occasional submucosal vessels in every control specimens.

The staining intensity of individual vessel walls and the number of positive vessels varied considerably in the inflamed specimens. Nevertheless the TCC positivity scores obtained from the two blind examinations were well correlated (t=7.7; p<0.001). Furthermore, there was a positive correlation between inflammatory score and vascular TCC deposits (t=8.05; p<0.001. Table 1).

In specimens from two UC patients we observed thrombosed submucosal blood vessels with TCC positivity both in the vessel wall and in the thrombus (Fig. 2). These arterioles were located beneath densely inflamed and damaged mucosa of patients who were severely ill when colectomy was performed.

Terminal complement complex deposits were detected on fibrillary elements (Fig. 3) in the muscularis mucosae or submucosa of five UC specimens (nos 4, 6, 7, 8, 9) and in one specimen from a

<table>
<thead>
<tr>
<th>Table 3</th>
<th>TCC deposits in control specimens without inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient no</td>
<td>Age (yrs)</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>29</td>
<td>87</td>
</tr>
<tr>
<td>30</td>
<td>51</td>
</tr>
<tr>
<td>31</td>
<td>57</td>
</tr>
<tr>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>33</td>
<td>56</td>
</tr>
<tr>
<td>34</td>
<td>86</td>
</tr>
<tr>
<td>35</td>
<td>78</td>
</tr>
<tr>
<td>36</td>
<td>78</td>
</tr>
<tr>
<td>37</td>
<td>70</td>
</tr>
<tr>
<td>38</td>
<td>80</td>
</tr>
</tbody>
</table>

**Table 2 Clinical information about patients with Crohn's disease in the ileum and TCC deposits in their ileal lesion**

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Medication</th>
<th>Topographic affection</th>
<th>Disease duration</th>
<th>Local inflam.</th>
<th>Vascular TCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>36</td>
<td>M</td>
<td>Pred.</td>
<td>C+I</td>
<td>UN</td>
<td>+/ +/ +/ +/ +</td>
<td>+/</td>
</tr>
<tr>
<td>25</td>
<td>26</td>
<td>M</td>
<td>UN</td>
<td>C+1</td>
<td>UN</td>
<td>+/</td>
<td>+/</td>
</tr>
<tr>
<td>24</td>
<td>18</td>
<td>M</td>
<td>No No</td>
<td>C+I</td>
<td>1 years</td>
<td>+/ +/ +/ +/ +</td>
<td>+/</td>
</tr>
<tr>
<td>26</td>
<td>43</td>
<td>M</td>
<td>No No</td>
<td>C+1</td>
<td>3 years</td>
<td>+/ +/ +/ +/ +</td>
<td>+/</td>
</tr>
<tr>
<td>27</td>
<td>25</td>
<td>F</td>
<td>Yes No</td>
<td>C+1</td>
<td>10 years</td>
<td>+/ +/ +/ +/ +</td>
<td>+/</td>
</tr>
<tr>
<td>28</td>
<td>40</td>
<td>M</td>
<td>UN UN</td>
<td>I</td>
<td>UN</td>
<td>+/</td>
<td>0/0</td>
</tr>
</tbody>
</table>

Pred. = prednisolone; Sala = salazopyrin; UN = unknown; C = colon; I = ileum. *The vascular TCC score from two blind examinations.
patient (no 18) with CD of the colon. Additional sections from these tissue blocks were re-examined and stained for elastin. The similarity of the staining patterns suggested that the TCC positivity was associated with the elastic fibrils. The majority of such fibrils, however, were TCC-negative. TCC-positive fibrils were not observed in histologically normal mucosa.

Germinall centres of secondary follicles from ileum (Peyer’s patches) and colon was positively stained for TCC in a dendritic pattern. This staining feature was shown to be associated with the follicular dendritic cells and could be observed in histologically normal mucosa and other lymphoid tissues as well.15

Discussion

The demonstration of substantially more TCC in colonic submucosal blood vessels in IBD than in histologically normal controls, is apparently a new observation of pathogenetic interest. There was no significant difference in vascular TCC deposits between UC and CD of the colon. The inflammatory lesions might in fact have contained more TCC than that observed, because we usually observed less vascular TCC positivity in paraffin blocks stored for more than 10 years. Eight of the UC, six of the colonic CD, and all the ileal CD blocks were older than 10 years, whereas none of the control blocks were older than six years.

The vascular TCC deposition should not be considered specific for IBD because trace amounts were seen in the controls, particularly with the sensitive APAAP method. Other workers have likewise observed immunohistochemical staining for TCC in arterioles and arteries in cryosections of normal human renal tissue26 and myocardium.17

Vascular complement deposits are often regarded as signs of a type III hypersensitivity reactions. Circulating immune complexes may become deposited in the vessel walls of inflamed areas. When rabbits with formalin induced rectal mucosal damage are injected intravenously with human serum albumin-anti-albumin immune complexes, the result is a severe colitis and crypt abscesses.26 Human endothelial cells have been shown to express receptors for C3b and Fcγ, in vitro when infected with herpes,27 cytomegalovirus or influenza virus.28 Bovine pulmonary endothelial cells express such receptors in vitro when incubated with white cell lysate.29 It is unknown whether inflammation per se will induce such receptors, but their expression may explain why circulating immune complexes preferentially become deposited in blood vessel walls of inflamed areas. It is controversial, however, whether patients with IBD have raised levels of circulating immune complexes.22

Other mechanisms could well be involved in the observed submucosal vascular TCC deposition. Locally produced IgG antibodies might complex with luminal antigens in situ and induce a local immune complex disease or Arthus type reaction as suggested by the presence of TCC-positive thrombosed submucosal blood vessels in two UC patients. Although vascular TCC deposits have been demonstrated in leucocytoclastic vasculitis of the skin,30 no cellular infiltrates or fibrinoid necrosis of the TCC-positive submucosal vascular walls were observed. The majority of our patients, however, received immunosuppressive medication which could have modified such reactions.

Fig. 2  Positive staining for TCC in a thrombosed submucosal vessel. Note TCC positivity both in the thrombus and in the vessel wall (arrows). APAAP staining on ethanol-fixed paraffin-embedded colonic specimens counterstained with haematoxylin from UC patients No. 7. (Magnification \( \times 100 \)).

Fig. 3  TCC positivity on fibrillary elements in muscularis mucosae (arrows). Identical TCC positive fibrils were also observed in the submucosa. APAAP staining of washed, ethanol-fixed and paraffin-embedded colonic specimens counterstained with haematoxylin from a patient with ulcerative colitis. (Magnification \( \times 162 \)).
TCC in inflammatory bowel disease

Previous immunohistochemical studies of complement factors in IBD have been based on antisera which do not distinguish between native and activated components. Also, tissue specimens with unpredictable amounts of retained extracellular proteins have been used. It is therefore uncertain whether previously observed Clq and C3 deposits in the intestinal epithelial basement membrane zone represented immune complexes or retained native components. We used extensively washed tissue specimens from which extracellular diffusible proteins had been extracted; and our MoAb to a neocrypteitope of TCC reacted exclusively with terminal activated complement. We observed virtually no TCC positivity in the basement membrane zone.

Mucosal terminal complement activation may, however, take place in the fluid phase. Although soluble immune complexes may induce complement activation and extensive C3 consumption, relatively little soluble TCC (SC5b-9) is produced. Furthermore, soluble SC5b-9 is most likely removed by our tissue washing procedure. We therefore believe that the vascular TCC was present in a tissue bound form and not as free soluble SC5b-9.

In addition to its well known biological effects, complement activation products such as C3a and C5a may trigger other biological systems that participate in the inflammatory reaction. C3b, iC3b, and C3c have been shown to stimulate human macrophages to release prostaglandin E2 (PGE2). and C3a and C3b induce thromboxane B2 (TXB2) release. C3a, C5a, and C5a des Arg have been shown to stimulate the production of leucotriene B4 in guinea pig ileum and in lung tissue cultures. Furthermore, generation of TCC on cell membranes have been shown to stimulate human macrophages to release PGE2 and TXA2. Such TCC also induces the production of free oxygen radicals by cultured rat mesangial cells. The possibility therefore exist that the increased amounts of TXB2 and PGE2 produced in vitro by rectal mucosa from UC and the raised concentrations of the PGE2 and leukotriene B4 shown by in vivo equilibrium dialysis of rectum in relapsing UC may have been induced by complement activation products. The TCC positive fibrillary elements observed in the muscularis mucosae and submucosa in five UC specimens and in one colonic CD specimen may have been associated with S-protein positive elastic fibrils as elastic fibres of human skin have been shown to stain for S-protein.

In conclusion, our results suggested that in situ terminal complement activation takes place in the intestinal lesions of UC and CD. Regardless of the mode of complement activation and the nature of the target attacked, generation of biologically active products (C3a, C5a, and TCC) will cause inflammation. Local terminal complement activation may therefore be of pathogenetic importance in IBD.

This work was presented in part at the XVII annual meeting of the Scandinavian Society for Immunology, Tampere, Finland, June 1986, and was published as an abstract in Scand J Immunol 1986; 24: 463. TSH is a Research Fellow of the Norwegian Cancer Society.

References

Deposits of terminal complement complex (TCC) in muscularis mucosae and submucosal vessels in ulcerative colitis and Crohn's disease of the colon.

T S Halstensen, T E Mollnes, O Fausa and P Brandtzaeg

Gut 1989 30: 361-366
do: 10.1136/gut.30.3.361

Updated information and services can be found at:
http://gut.bmj.com/content/30/3/361

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
- Crohn's disease (932)
- Ulcerative colitis (1113)
- Colon cancer (1547)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/