Immunity to cytopathic agents associated with Crohn’s disease: a negative study

M CHIBA, L C MCLAREN, AND R G STRICKLAND*
From the Division of Gastroenterology, Department of Medicine, University of New Mexico, School of Medicine, Albuquerque, New Mexico, USA

SUMMARY Serum and peripheral blood lymphocytes from 10 patients with Crohn’s disease and 10 healthy subjects were examined for immunological reactivity against chick embryo cell cultures displaying cytopathic effects after inoculation with 0·2 μ filtrates prepared from Crohn’s disease intestinal tissues. Although the assay systems (indirect immunofluorescence, lymphocyte transformation, and cytotoxicity) yielded positive results using well-characterised cytopathic viruses (mumps, measles), neither Crohn’s disease nor healthy subjects showed immune reactivity to the chick embryo cell cultures inoculated with Crohn’s disease intestinal tissues in any of the assay systems. These experiments provide evidence against the hypothesis that the in vitro cytopathic effect on chick embryo cell cultures produced by Crohn’s disease intestinal filtrates are caused by a replicating virus or viruses.

Independent studies from three laboratories have shown that 0·2 μ filtrates of intestinal homogenates from patients with Crohn’s disease cause transmissible cytopathic effects when inoculated into a variety of low passage fibroblast-like cell cultures.1-3 The frequency of this in vitro abnormality using Crohn’s disease intestine (80–90%) is significantly higher than that seen using disease control intestinal preparations (25%).3

Early studies of the properties of this cytopathic effect suggested that it was produced by an RNA-virus.1-3 More recent work by Phillpotts et al., however, has questioned these findings4 and subsequently indicated that a soluble toxin(s) may be responsible for the in vitro cytopathic effect.5 6

In the present studies, we have used an immunological approach in attempting to clarify further the nature of the cytopathic effects induced by Crohn’s disease tissue filtrate (CD-TF) in cell culture. We hypothesised that if these effects were viral in nature, viral antigens would be expressed in such cultures and host immune reactivity to these antigens should be demonstrable.

Methods

Patients

Venous blood was obtained from 10 patients with Crohn’s disease (six women, four men) with a mean age 35 years (20–63 years) and 10 healthy adults (five women, five men) with a mean age of 29 years (23–39 years). Among the patients with Crohn’s disease six had ileitis, two had ileocolitis, and two had colitis. Eight of the 10 patients had had the disease for more than five years. At the time of blood sampling four patients had active disease, five inactive, and one post-ileal resection two days before. Five patients were taking no drugs at the time of study. The remaining five patients were receiving sulphasalazine (one), sulphasalazine and prednisone (one), or prednisone (three). Serum was separated from an aliquot of the venous sample and stored at −70°C. The remainder of the sample was heparinised and used for lymphoid cell isolation.

Crohn’s disease intestinal filtrate preparation (CD-TF)

Bacteria-free and mycoplasma-free 0·2 μ filtrates were prepared from fresh surgical samples of affected intestine.2 7 8 Tissue filtrates from five different patients with Crohn’s disease were selected for the present studies on the basis of their production of virus-like cytopathic effects in chick embryo cells.
Tissue culture, CD-TF inoculation, and observation of cytopathic effects

As chick embryo cells were the most sensitive to the filtrate preparation, most of the immunological experiments were done using these culturertarget cells. Cell culture growth and maintenance media were the same as described by Gitnick et al. Chick embryo cultures were established from 10-day-old fertile eggs (SPAFAS, Chicago, Ill). Passage two-chick embryo cells at 2×10²/200 µl growth medium were seeded into the covers of flat bottom microtitre plates (3040 Falcon, Oxnard, Ca). When monolayers were confluent, the medium was removed and the cells were incubated with 50 µl of the filtrate or 50 µl of Hanks balanced salt solution (HBSS) control for one hour at room temperature. Then 150 µl of maintenance medium was added, and the monolayers were incubated at 37°C in humidified 5% CO₂ air. The monolayers were observed for the development of cytopathic effects after the inoculation and scored as follows: − cell appearance similar to control monolayer; + a focal cell detachment over a very small area; ++ definite rounding up cells, with or without cell detachment, over less than half of the well area; +++ cell detachment over more than half of the well area. The same procedure for inoculation and scoring of cytopathic effects was observed in cultures used as positive virus controls. These included chick embryo cells inoculated with mumps virus (Enders strain) at a titre of 10⁵–10⁶ TCID₉₀/ml and Vero cells (American Type Culture Collection, Rockville, Md), inoculated with measles virus (Edmonson strain, American Type Culture Collection) at a titre of 5×10⁴ PFU/ml.

Isolation of peripheral blood lymphoid cells

Heparinised venous blood was diluted 1:2 with phosphate buffered saline and the lymphoid cells were isolated by Ficoll-Hypaque (Pharmacia, Fine Chemicals, Piscataway, NJ) density gradient centrifugation. Cell viability was over 95% by trypan blue exclusion, and the preparations contained 5–25% monocytes as judged by benzidine staining.

Immunological studies

Lymphocyte transformation

Lymphocyte transformation by glutaraldehyde-fixed monolayers Chick embryo monolayers displaying ++ – + +++ cytopathic effects after inoculation with the filtrate preparation or control monolayers were used as targets for lymphocyte transformation by a modification of the method of McFarland et al. Monolayers in flat bottom microtitre plates were fixed with 0-005% glutaraldehyde (Sigma, St. Louis, Mo) for 30 minutes at 4°C and washed three times with phosphate buffered saline. Then 2×10⁵ peripheral blood lymphoid cells in 150 µl RPMI 1640, FCS₁₀PS were added per well. After four days' incubation, the cells were pulsed for six hours with 0-36 µCi [³H]-thymidine (6-7 Ci/mmol, New England Nuclear, Boston, Mass) per well, harvested with a multiple automated sample harvester, and counted. Uptakes of [³H]-thymidine were expressed as mean cpm ± one standard error as calculated from quadruplicate samples. Mumps virus (Enders) was used as a positive virus control in this experiment.

Lymphocyte transformation in suspension Crohn’s disease intestinal filtrate preparations neat and at dilutions up to 1:8 were used as potential stimulants for 2×10⁵ peripheral blood lymphoid cells in 150 µl RPMI 1640, FCS₁₀PS per well in round bottom type microtitre plates (Nunclon, Nuncatom, Roskilde, Denmark). Additional experiments were carried out using cell culture extracts as potential lymphocyte stimulants as described for human herpes virus infection. After the cytopathic effect was manifested, the monolayer was scraped off with a rubber policeman into 0.5 ml Hanks balanced salt solution or 0-043M glycine buffered saline and then subjected to freeze-thawing. Supernatants were collected after centrifugation at 600 g for 10 minutes. As a negative control, extracts of uninoculated monolayers were prepared in the same manner. Fifty microlitres of the supernatant were added to 2×10⁵ peripheral blood lymphocytes in 150 µl RPMI 1640, FCS₁₀PS in round bottom wells. After six days' incubation, the lymphocytes were pulsed with [³H]-thymidine and harvested as described for glutaraldehyde fixed monolayers.

Lymphocyte-mediated cytotoxicity Chick embryo cell monolayers grown in flat bottom microtitre wells—either uninoculated or inoculated with the Crohn’s disease intestinal filtrate preparation—were incubated with medium containing 0.5 µCi of [⁵¹Cr] Na₂ CrO₄ (50–400 m Ci/mg Cr, American Corp., Arlington Heights, Ill) for six hours, then washed three times with Eagle’s minimal essential medium. 1×10⁶ peripheral blood lymphocytes in 200 µl RPMI₁₀FCS₁₀PS were added to each well, and, after 16 hours' incubation, a 100 µl aliquot was carefully taken into a tube from each well without disturbing the monolayer in the bottom and replaced with 100 µl of diluted (1:10) Zaponin solution (Coulter Diagnostics Inc., Hialeah, Fla) to lyse the target cells. After an additional 24 hours' incubation, another 100 µl aliquot was taken. These aliquots were counted in a well-type gamma scintillation counter (Packard, Downers Grove, Ill). The percent-
Immunity to cytopathic agents associated with Crohn's disease

age of $^{51}$Cr release was calculated by the following method:

$$\text{%}^{51}\text{Cr release} = \frac{\text{released}^{51}\text{Cr in medium} \times 100}{\text{total releasable}^{51}\text{Cr} \times b + \frac{1}{2} a}$$

where $a = \text{cpm obtained from the first aliquot of 100 \mu l}$

$b = \text{cpm obtained from the second aliquot of 100 \mu l}$

This method, in which total releasable $^{51}$Cr was obtained for each well tested, permits the calculation of an accurate %$^{51}$Cr release. Spontaneous $^{51}$Cr release distributed between 25% and 35%. Spontaneous cell mediated cytotoxicity (SCMC) was obtained by the following calculation: SCMC(%) = %$^{51}$Cr release obtained from the incubation of peripheral blood lymphocytes and the inoculated or uninoculated monolayer—spontaneous release from the corresponding monolayer. Results were expressed as the mean of the quadruplicates ± one standard error. Measles virus was used as a positive virus control in this experiment.

### INDIRECT IMMUNOFLUORESCENCE ANTIBODY TEST

Chick embryo cells, passage 2, were seeded to Lab-tek Tissue culture chamber/slides (No. 4808, Naperville, Ill) and cultured until they were a confluent monolayer. The monolayers were inoculated with 50 \mu l of the intestinal filtrate preparation, and, after one hour, 250 \mu l of maintenance medium was added to the chamber. After the appearance of the cytopathic effect, the monolayers were washed with phosphate buffered saline, dried, then fixed with cold acetone for 10 to 15 minutes followed by washing with the saline. Sera from patients with Crohn's disease, healthy subjects, or persons in the acute (CF titre 1:16) or convalescent (CF titre 1:256) stages of measles were tested for antibody by the standard indirect method, using both FITC conjugated goat antimouse immunoglobulins (Antibody Incorporated, Davis, Ca) or FITC conjugated antihuman 1 gM (Cappel Lab, Cochranville, Pa) as the second antibody.

### Results

#### LYMPHOCYTE TRANSFORMATION

Lymphocyte transformation by glutaraldehyde-fixed monolayers (Table 1) $[^{3}H]$-thymidine uptake by fixed monolayers alone was negligible (<50 cpm). Peripheral blood lymphoid cells from all nine patients with Crohn's disease and 10 healthy controls showed the expected response to PHA. Those cells from two adults with a previous history of mumps showed substantial transformation in response to the mumps inoculated fixed

<table>
<thead>
<tr>
<th>Donor</th>
<th>Disease</th>
<th>Unstimulated</th>
<th>PHA (2.5 \mu g/ml)</th>
<th>Uninoculated monolayer</th>
<th>CD-TF inoculated monolayer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT</td>
<td>CD</td>
<td>596±135</td>
<td>6396±267</td>
<td>391±78</td>
<td>367±77</td>
</tr>
<tr>
<td>IN</td>
<td>CD</td>
<td>443±240</td>
<td>25880±1575</td>
<td>466±141</td>
<td>387±24</td>
</tr>
<tr>
<td>MG</td>
<td>CD</td>
<td>2111±94</td>
<td>33774±717</td>
<td>1390±141</td>
<td>2034±149</td>
</tr>
<tr>
<td>MN</td>
<td>CD</td>
<td>1547±40</td>
<td>4437±576</td>
<td>1482±277</td>
<td>1597±328</td>
</tr>
<tr>
<td>AG</td>
<td>CD</td>
<td>763±62</td>
<td>26168±2319</td>
<td>999±51</td>
<td>1235±31</td>
</tr>
<tr>
<td>FR</td>
<td>CD</td>
<td>323±51</td>
<td>21342±613</td>
<td>353±9</td>
<td>361±38</td>
</tr>
<tr>
<td>CB</td>
<td>CD</td>
<td>604±126</td>
<td>21247±4709</td>
<td>1269±122</td>
<td>1570±194</td>
</tr>
<tr>
<td>WM</td>
<td>CD</td>
<td>1024±52</td>
<td>28190±868</td>
<td>954±46</td>
<td>1420±11</td>
</tr>
<tr>
<td>JS</td>
<td>CD</td>
<td>1173±89</td>
<td>23230±1709</td>
<td>903±265</td>
<td>1174±90</td>
</tr>
<tr>
<td>JE</td>
<td>Control</td>
<td>2304±331</td>
<td>14274±2276</td>
<td>2225±267</td>
<td>2721±322</td>
</tr>
<tr>
<td>ST</td>
<td>Control</td>
<td>1185±73</td>
<td>46587±3485</td>
<td>909±105</td>
<td>1286±14</td>
</tr>
<tr>
<td>AN</td>
<td>Control</td>
<td>2329±411</td>
<td>16008±743</td>
<td>1939±180</td>
<td>2010±208</td>
</tr>
<tr>
<td>MC</td>
<td>Control</td>
<td>1123±97</td>
<td>23526±1062</td>
<td>1161±61</td>
<td>1210±58</td>
</tr>
<tr>
<td>LZ</td>
<td>Control</td>
<td>679±201</td>
<td>23135±1234</td>
<td>5924±632</td>
<td>6374±482</td>
</tr>
<tr>
<td>RO</td>
<td>Control</td>
<td>4398±718</td>
<td>47002±1291</td>
<td>9247±829</td>
<td>12642±545</td>
</tr>
<tr>
<td>DA</td>
<td>Control</td>
<td>2320±187</td>
<td>54130±5628</td>
<td>3024±400</td>
<td>2932±398</td>
</tr>
<tr>
<td>LO</td>
<td>Control</td>
<td>5693±1169</td>
<td>75813±8127</td>
<td>6280±850</td>
<td>8099±1353</td>
</tr>
<tr>
<td>LI</td>
<td>Control</td>
<td>1816±158</td>
<td>11639±456</td>
<td>2904±72</td>
<td>2451±328</td>
</tr>
<tr>
<td>PA</td>
<td>Control</td>
<td>6465±2278</td>
<td>17840±1708</td>
<td>5411±889</td>
<td>6131±532</td>
</tr>
</tbody>
</table>

Mumps virus inoculated monolayer

| LO    | Healthy adults with history of mumps | 2932±516 | 63012±5061 | 6140±1032 | 31138±5320 |
| OK    | 1873±201 | 16549±1209 | 1595±181 | 4050±68 |

Values indicate mean $[^{3}H]$-thymidine uptake (CPM)±SEM of quadruplicate samples.
monolayer showing ++ cytopathic effects when compared with uninoculated monolayers. By contrast, the peripheral blood lymphoid cells from the patients with Crohn's disease and from the healthy controls showed no response to the filtrate inoculated fixed monolayers showing equivalent cytopathic effect to that induced by mumps virus. Crohn's disease intestinal filtrate preparations from three different patients were used in these experiments, and each lymphocyte donor was tested against at least two different filtrate inoculated monolayers. Peripheral blood lymphocytes from two patients with Crohn's disease and four controls were harvested on the fourth, fifth, and sixth days of incubation with the cell monolayers, and again no response was observed.

**Lymphocyte transformation by Crohn's disease tissue filtrate and cell culture extracts**

This filtrate when used undiluted significantly inhibited [³H]-thymidine uptake by the peripheral blood lymphocytes from four patients with Crohn's disease and four healthy subjects (Table 2). In four subjects tested, the inhibitory effect was substantially decreased by serial dilution of the filtrate (Table 2). In three subjects (one Crohn's disease, two controls), all five filtrates were tested. Spontaneous [³H]-thymidine uptake in these three individuals was uniformly inhibited by all five filtrates. This inhibitory effect of the Crohn's disease tissue filtrate was not eliminated by heat treatment (65°C, 20 minutes) or by ultraviolet irradiation. The responses of four patients with Crohn's disease and one healthy control were not altered by incubation with GBS cell extracts derived from two filtrate inoculated cultures or to the same cell extracts without GBS treatment (Table 3).

**Lymphocyte-mediated cytotoxicity**

Peripheral blood lymphoid cells from 10 patients with Crohn's disease and nine healthy subjects were each tested against at least two monolayers inoculated with Crohn's disease tissue filtrates. The lymphoid cells from most donors showed some spontaneous cell mediated cytotoxicity over that due to spontaneous release. There was, however, no difference in this cytotoxicity in inoculated compared with uninoculated monolayers (Table 4). By contrast, using the same assay system, lymphoid cells from healthy subjects with a history of measles showed higher cytotoxicity against vero cells infected with measles virus than against uninfected vero cells (Table 4).

**Indirect immunofluorescence antibody test**

Ten sera from patients with Crohn's disease and 10 sera from healthy subjects were tested at a 1:10 dilution against monolayers of chick embryo cells inoculated with two different Crohn's disease tissue filtrates and showing + + + + cytopathic effect. In both uninoculated and Crohn's disease tissue filtrate inoculated preparations, the sera from healthy subjects and patients with Crohn's disease reacted with monolayers of chick embryo cells inoculated with filtrates from all 10 patients. The sera from healthy subjects reacted to monolayers of chick embryo cells inoculated with filtrate from the same patients with Crohn's disease.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Disease</th>
<th>No. of CD-TF tested</th>
<th>Unstimulated</th>
<th>Stimulated with CD-TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>CD</td>
<td>2</td>
<td>152±21</td>
<td>62±6</td>
</tr>
<tr>
<td>IN</td>
<td>CD</td>
<td>2</td>
<td>1176±180</td>
<td>287±118</td>
</tr>
<tr>
<td>BT</td>
<td>CD</td>
<td>1</td>
<td>283±143</td>
<td>79±18</td>
</tr>
<tr>
<td>GM</td>
<td>CD</td>
<td>5</td>
<td>296±79</td>
<td>39±9</td>
</tr>
<tr>
<td>PA</td>
<td>Control</td>
<td>5</td>
<td>420±51</td>
<td>41±19</td>
</tr>
<tr>
<td>MC</td>
<td>Control</td>
<td>5</td>
<td>343±53</td>
<td>47±15</td>
</tr>
<tr>
<td>JE</td>
<td>Control</td>
<td>2</td>
<td>1030±219</td>
<td>418±72</td>
</tr>
<tr>
<td>ST</td>
<td>Control</td>
<td>2</td>
<td>2040±264</td>
<td>942±192</td>
</tr>
</tbody>
</table>

**Table 2 Peripheral blood lymphocyte transformation in response to Crohn's disease tissue filtrates (CD-TF)**

NT: not tested.
Values indicate mean [³H]-thymidine uptake (CPM)±SEM of quadruplicate samples.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Disease</th>
<th>Unstimulated</th>
<th>Extract</th>
<th>Tissue culture extract stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN</td>
<td>CD</td>
<td>448±122</td>
<td>GBS</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>MG</td>
<td>CD</td>
<td>487±79</td>
<td>GBS</td>
<td>Inoculated with CD-TF¹</td>
</tr>
<tr>
<td>MC</td>
<td>Control</td>
<td>1092±41</td>
<td>GBS</td>
<td>1663±244</td>
</tr>
<tr>
<td>AG</td>
<td>CD</td>
<td>1223±204</td>
<td>Non-GBS</td>
<td>1190±370</td>
</tr>
<tr>
<td>FR</td>
<td>CD</td>
<td>2003±217</td>
<td>Non-GBS</td>
<td>2542±241</td>
</tr>
</tbody>
</table>

**Table 3 Peripheral blood lymphocyte transformation in response to GBS and non-GBS extracts of cell cultures inoculated with Crohn's disease tissue filtrates (CD-TF)**

Values indicate mean [³H]-thymidine uptake (CPM)±SEM of quadruplicate samples.
inoculated monolayers all sera caused faint, diffuse cytoplasmic staining. Neither an increase in the intensity of the fluorescent staining nor an increase in the number of stained cells was seen in monolayers inoculated with the filtrate. Serial serum dilutions (1:5–1:60), the use of goat anti-IgM as second antibody or the use of another cell culture system susceptible to the Crohn’s disease filtrate (WI-38) did not result in significant fluorescent reactions. By contrast, in the virus control experiment, sera (1:10–1:60 dilutions) from two patients with mesas in either the acute or convalescent stage showed bright staining against mesas infected vero cells and no staining against uninfected control monolayers.

Discussion

Previous studies of the in vitro cytopathic effect of Crohn’s disease intestinal filtrates have suggested that it is caused by an RNA-virus associated with the diseased bowel of such patients.\(^1\)\(^2\)\(^3\) Thus, the cytopathic effect is transmissible in cell culture, unaffected by heat, ether, acid, or IUdR and virus-like particles have been described in cultures undergoing this effect.\(^1\)\(^2\)\(^3\) If this effect is caused by a cytopathic virus, then, by analogy with other known virus systems, host immunity to the agent should be readily demonstrable. Thus in the present studies we have used several established techniques in attempting to show cellular and humoral immunity to putative virus(es) present in cell cultures undergoing cytopathic effect after inoculation with Crohn’s disease intestinal filtrates.

Glutaraldehyde-fixed cell monolayers displaying cytotoxicity after inoculation with mumps, vaccinia, and measles viruses have been shown to elicit lymphocyte transformation, using peripheral blood lymphoid cells from subjects previously sensitised to these viruses.\(^1\)\(^1\) In the present study we have confirmed this finding using mumps virus infected fixed cell monolayers. Using identical cell monolayers undergoing cytopathic effect in response to inoculation with Crohn’s disease intestinal filtrates, however, no transformation of lymphoid cells from patients with Crohn’s disease or healthy controls was observed (Table 1).

We also attempted to elicit lymphocyte transformation in suspension using the Crohn’s disease intestinal filtrate and cell extracts from cultures undergoing cytopathic effect after inoculation with this filtrate. The use of these extracts was predicted on evidence indicating that cell membrane–associated viral antigens are of key importance in the production of in vitro lymphocyte transformation.\(^1\)\(^4\)\(^5\) Such extracts have been successfully used to demonstrate lymphocyte transformation in human herpes virus infection.\(^1\)\(^2\)\(^3\) In the present study, the undiluted filtrate actually caused consistent inhibi-
tion of lymphocyte transformation, and the filtrate
inoculated cell extracts yielded no response either
stimulatory or inhibitory (Tables 2, 3).

The in vivo significance of in vitro lymphocyte-
mediated cytotoxicity against virus infected target
cells remains uncertain. Nevertheless, this in vitro
assay has been shown to reflect lymphocyte (T-cell,
N-K cell, or both) reactivity in several established
viral infections in man. Using peripheral
blood lymphoid cells from subjects exposed to
measles, we have confirmed the presence of en-
hanced cytotoxicity against cells cultured infected
with measles virus. By contrast, using these cells
from control subjects or patients with Crohn’s disease in a
similar assay system incorporating cell cultures in-
oculated with Crohn’s disease intestinal filtrates, no
enhanced cytotoxicity was demonstrable (Table 4).
As these experiments used xenogeneic target cells,
the major function measured by these assays was
probably that of N-K activity, rather than T-cell
cytotoxicity.

Our previous attempts to detect antibody to the
putative viral agents in Crohn’s disease necessitated
serum neutralisation of the cytopathic effect. These
studies showed inhibition only at low serum dilu-
tions, and no differences were observed between sera
from patients with Crohn’s disease and normal sera.
The present results using immunofluorescence also
indicate the absence of apparent antibody activity in
sera from patients with Crohn’s disease or normal sera
against antigens expressed in cell cultures
undergoing the cytopathic effect after inoculation
with Crohn’s disease intestinal filtrates. These
findings are in agreement with those recently re-
ported by Phillpotts et al.

Thus, using established methods for detecting
cellular and humoral immunity to known cytopathic
viruses in culture, we have been able to show such
reactivity to the proposed agents associated with
Crohn’s disease previously implicated in causing in vitro
cytopathic effects. The number of donors
studied (10 with Crohn’s disease, 10 controls) was not
large, but the consistency of the findings makes sub-
ject selection an unlikely explanation for the negative
results. Could the findings be attributable to a more
generalised depression of cellular immune function
in Crohn’s disease? Previous studies of cellular
immunity in Crohn’s disease have been in sharp conflict
with regard to the presence or absence of T-cell
deficiency. Moreover, in the present study there was
no significant difference in PHA responses between
control or Crohn’s disease PBL. Recently, N-K acti-

ivity has been reported to be reduced in Crohn’s
disease, and possibly our negative findings using
cytotoxicity could reflect deficient N-K function. This


possibility would not, however, explain the negative
results obtained using transformation assays.

In conclusion, we suggest that the results of the
present study raise considerable doubt about the
proposed viral causation of the in vitro cytopathic
effect induced by Crohn’s disease tissue filtrates.
Very recent studies by Phillpotts et al. and
McLaren et al. also provide evidence against this
proposal. These workers have shown that many of
the properties of the cytolysis induced by these
filtrates are more consistent with a tissue associated
toxic effect. Indeed, the inhibitory effect of Crohn’s
disease tissue filtrates on lymphocyte function
observed in the present study may also represent such a
toxic effect.

We thank Elizabeth Dow for technical assistance and
Mr R Steece of New Mexico State Health Laboratory
for providing the mumps virus used in this study. This
work was supported by USPHS Research Grant
AM27354.

References
1 Aronson MD, Phillips CA, Beeken WL, Forsyth BR.
Isolation and characterisation of a viral agent from intes-
tinal tissue of patients with Crohn’s disease and other
2 Gitnick GL, Arthur MN, Shibata I. Cultivation of viral
agents from Crohn’s disease: A new sensitive system.
3 Strickland RG, McLaren LC. Studies of the in vitro
cytopathic effect of inflammatory bowel disease tissue
preparations. In: Pena AS, Weterman IT, Booth CC,
Strober W, eds. Developments in gastroenterology. The
4 Phillpotts RJ, Hermon-Taylor J, Brooke BN. Virus
isolation studies in Crohn’s disease: a negative report.
5 Phillpotts RJ, Hermon-Taylor J, Teich NM, Brooke BN.
A search for persistent virus infection in Crohn’s dis-
6 Phillpotts RJ, Hermon-Taylor J, Brooke BN. Evidence
against the involvement of conventional viruses in
Crohn’s disease. In: Pena AS, Weterman IT, Booth CC,
Strober W, eds. Developments in gastroenterology. The
7 Gitnick, Rosen VJ, Arthur MH, Hertweck, SA. Evi-
dence for the isolation of a new virus from ulcerative
colitis patients: comparison with virus derived from
Crohn’s disease. Digestive Disease and Science 1979; 24:
609–19.
8 Hayflick L. Tissue culture and mycoplasmas. Texas Rep
9 Aiuti F, Cerottini JC, Coombe RA, et al. Special tech-
nical report. Identification, enumeration and isolation
of B and T lymphocytes from human peripheral blood.
10 Kaplow LS. Simplified myeloperoxidase stain using ben-
Immunity to cytopathic agents associated with Crohn's disease
