Experimental induction of rheumatoid factor and joint lesions in rabbits after intravenous injections of killed bacteria

A C HANGLOW,¹ C J R WELSH,¹ P CONN,² J M PITTS,¹ A RAMPLING,³ AND R R A COOMBS¹

From the ¹Department of Immunology, Department of Pathology, University of Cambridge; the ²Department of Histopathology, Addenbrooke’s Hospital, Cambridge; and the ³Public Health Laboratory Services, Addenbrooke’s Hospital, Cambridge

SUMMARY  Rabbits receiving repeated intravenous injections of killed bacteria (Escherichia coli or Bacillus subtilis) developed IgM rheumatoid factor which reacted with autologous heat-aggregated IgG. In addition, 5/7 ‘Old English’ and 7/8 ‘Sandy Lop’ rabbits receiving killed E. coli developed rheumatoid-like synovial lesions. ‘Old English’ rabbits developed lesions of a more severe nature. Three of eight ‘Sandy Lop’ rabbits injected with killed B. subtilis had high levels of rheumatoid factor but only mild joint lesions.

Key words: arthritis, autologous heat-aggregated IgG, Escherichia coli, Bacillus subtilis.

Rheumatoid factor-like substances (RFLS) have been described in rabbits after the administration of a variety of bacterial¹ or protein²³ antigens. However, joint lesions were not reported in any of these models. In this paper we report the induction of both rheumatoid factor (RF), reacting with autologous heat-aggregated IgG, and rheumatoid-like synovial lesions in rabbits injected with either killed Escherichia coli or Bacillus subtilis.

Materials and methods

RABBITS

‘Old English’ rabbits were purchased from Cheshire Rabbit Farms Ltd, Dudden Lodge, Cheshire, UK. ‘Sandy Lop’ rabbits were purchased from the Medical Research Council. All animals received food (R14 diet, K K Greeff Ltd, Croydon, UK) and water ad libitum.

PREPARATION OF Escherichia coli AND Bacillus subtilis

Escherichia coli (E. coli NCTC 9016) and Bacillus subtilis (B. subtilis NCTC 3610) were grown in 1 litre volumes in 3 litre Erlenmeyer flasks at 37°C for 48 hours in brain/heart infusion broth (Difco). Formalin (final concentration of 0-6%) was then added to the broth cultures, which were left for 24 h at 20°C in order to kill the bacteria. The bacteria were harvested by centrifugation, and the deposit was washed three times in phosphate-buffered saline (PBS). The bacteria were resuspended in PBS and standardised by opacity testing to 6 × 10⁹ bacteria/ml. The bacteria were finally sterilised by steaming for 1 h and thereafter stored at −20°C.

INJECTION PROTOCOL

‘Old English’ and ‘Sandy Lop’ rabbits received repeated intravenous injections of killed E. coli (2 × 10⁹ bacteria/0·3 ml injections three times weekly) over a period of 12 weeks. In addition, another group of ‘Sandy Lop’ rabbits were injected with killed B. subtilis under an identical regimen. Control ‘Old English’ and ‘Sandy Lop’ rabbits did not receive any bacteria. Rabbits were bled at monthly intervals from the marginal vein of the ear. Sera were frozen and stored at −20°C.

ASSESSMENT OF ARTHRITIS

Rabbits were killed by lethal administration of sodium pentabarbitone (May and Baker Ltd). Syno-
vial fluids were recovered as previously described, centrifuged at 200 g for 15 min at 4°C, and the supernatants removed and stored at −20°C. Synovial fluid cells were resuspended in balanced salt solution (BSS, Flow Laboratories) supplemented with 1% heat-inactivated fetal calf serum (Flow Laboratories).

**Enumeration of nucleated cells in synovial fluid**
The number of viable nucleated cells in the synovial fluids of rabbits was estimated with a mixture of acridine orange and ethidium bromide. Cells were usually more than 95% viable.

**Enumeration of T cells in synovial fluids**
The percentage of T cells in synovial fluids was determined by rosetting with papain-treated rabbit erythrocytes as previously described.

**Dissection of joints and histological procedures**
Both knee joints from each rabbit were dissected and the synovia divided into infrapatellar, interpatellar, and lateral synovia before fixing in 10% neutral buffered formalin and embedding in paraffin. 5 μm sections were cut, stained with haematoxylin and eosin, and graded for inflammatory lesions as previously described.

**Kidney histology**
Kidney samples were obtained after death, fixed in formalin, and embedded in paraffin. 3 μm sections were cut and stained conventionally with haematoxylin and eosin and with the standard periodic acid silver technique.

**Measurement of IgM Rheumatoid Factor**
In order to overcome the problem of naturally occurring antiallootypic antibodies in rabbits serum RFs were measured by their ability to bind to autologous heat-aggregated IgG. Before the start of the experiment IgG was prepared from the serum of each rabbit by the caprylic acid method. Rabbit IgG (1 g/l in normal saline) was aggregated by heating at 70°C for 10 min and stored at −20°C. RF was measured in the Mrs PAH test (mixed reverse (solid phase) passive antiglobulin haemadherence) described by March et al. Briefly, microtitre plates (U shaped) (Sterilin, Teddington) were coated overnight at 4°C with 60 μl of a 10 mg/l solution of heat-aggregated rabbit IgG diluted in carbonate coating buffer (0-05 M, pH 9-6). Sera and synovial fluids were serially diluted in PBS+0-08% Tween 20 diluent, and 60 μl of each dilution was added to the coated plates. After incubation for 90 min at 4°C the plates were washed three times with cold PBS/Tween and subsequently three times with PBS. IgM RF binding to autologous IgG was detected with a goat anti-rabbit μFc (a gift from Dr A Feinstein) coupled by chromic chloride to trypsin-treated sheep red blood cells. One drop of a 1% suspension of these indicator cells was added to each well of the microtitre plates. The plates were agitated and read for haemagglutination after the erythrocytes had settled.

**Measurement of Serum Antibodies to E.coli and B.subtilis**
Serum antibodies to *E.coli* and *B.subtilis* were measured in a solid phase binding assay. Briefly, a soluble bacterial antigen was prepared by sonication of a suspension of killed *E.coli* or *B.subtilis* (6 × 10⁹ bacteria/ml) in a cup-horn sonicator (Heat Systems 10375) by pulsing until visible disruption of the bacterial suspension occurred (5–10 min at 50% duty cycle). The bacterial extract was centrifuged (11 500 g) and the supernatant adjusted to 100 mg protein/l (absorbance read at 280 nm) in disodium carbonate buffer (50 mM, pH 9-6 containing 0-002% sodium azide). Each well of a polyvinyl U well microtitre plate (Falcon Microtest III flexible assay plate) was coated with 50 μl of the bacterial preparation by incubating overnight at 4°C. The plates were washed with PBS containing 0-05% Tween 20 and then coated with blocking buffer (PBS containing 1% bovine serum albumin, 1% normal sheep serum, and 0-1% sodium azide) by incubating at 37°C for 60 min. After washing 50 μl of serial dilutions of test sera were added to the plate and incubated for two hours at 20°C. After further washing antibodies to *E.coli* or *B.subtilis* were detected by the addition of 50 μl of a 1% suspension of trypsin-treated sheep erythrocytes coupled to IgG goat antirabbit γFc by the chromic chloride method (anti-γFc a gift from Dr A Feinstein). The plates were agitated and read for haemagglutination after the erythrocytes had settled.

**Statistical Analysis**
This was performed by the Student’s *t* test on log₂ transformed data.

**Results**

**Detection of Serum IgM Rheumatoid Factor and IgG Antibacteria Antibodies**
Measurement of serum IgM rheumatoid factor
The highest titres of serum IgM RF were detected in ‘Sandy Lop’ rabbits injected with killed *B.subtilis* (mean titre±SD 42±3) compared with either ‘Sandy Lop’ or Old English’ rabbits injected with killed...
E. coli (mean titres ± SD of 14±3 and 5±2 respectively; p values of <0.05 and <0.001 respectively). Uninjected rabbits had low serum titres of IgM RF, in most cases this was a titre of <4 (Fig. 1).

Measurement of serum antibodies to E. coli and B. subtilis
In addition to being the highest producers of RF ‘Sandy Lop’ rabbits injected with B. subtilis also had the highest serum antibody IgG responses to the injected bacteria (p < 0.001 compared with both ‘Sandy Lop’ and ‘Old English’ rabbits injected with E. coli) (Fig. 2). There was not a significant difference between serum antibody IgG responses to E. coli in either ‘Old English’ or ‘Sandy Lop’ rabbits.

Fig. 1 The development of IgM RF in rabbits repeatedly injected intravenously with killed bacteria: 'Old English' rabbits received injections of killed E. coli (2 × 10⁸/injection three times weekly for 12 weeks). 'Sandy Lop' rabbits received either killed E. coli or B. subtilis under an identical regimen. Serum IgM RF levels were measured after death by their ability to bind to heat-aggregated autologous IgG (as described in 'Materials and Methods'). Statistical analysis was performed by the Student's t test on log₂ transformed data.

Abuzzo and Christian¹ proposed that prolonged immunisation with killed bacteria resulted in the continued formation and elimination of immune complexes which stimulated the production of RFIS with specificity for the animal's own IgG when aggregated or as part of an immune complex. In order to investigate this possibility serum IgM RF levels were plotted against serum IgG antibody levels against the injected bacteria (Fig. 2). High levels of IgM RF were associated in most injected rabbits with high levels of serum IgG antibody reacting with the injected bacteria, though this association was not statistically significant. High IgM RF levels were not associated with high levels of specific IgM antibody in any of the experimental groups (data not shown). This suggests that the IgM RF does not cross react with the injected microorganism. It, therefore, seems most probable that the serum IgM RF is directed against IgG present in immune complexes containing bacterial antigens. The low levels of serum IgM RF detected in control animals may be directed against IgG reacting with commensal microorganisms.

Development of joint lesions in rabbits injected with killed bacteria
Incidence of lesions
Repeated intravenous injections of heat-killed E. coli induced rheumatoid-like synovial lesions in 5/7
Induction of rheumatoid factor and joint lesions in rabbits

(71%) 'Old English' and 7/8 (88%) 'Sandy Lop' rabbits (Table 1). However, all the lesions observed in 'Sandy Lop' rabbits were only mild in nature, whereas 3/7 (43%) of the 'Old English' rabbits developed lesions classified as moderate to severe. Three out of eight 'Sandy Lop' rabbits injected with killed B. subtilis developed joint lesions which were all classified as 'mild'. One untreated female 'Sandy Lop' rabbit developed a slight infiltration of lymphocytes in one synovium. This reflects the low incidence of spontaneous arthritis in this strain of 14% (1/7). None of the control 'Old English' rabbits used in this series of experiments developed synovial changes, though previous work has shown the spontaneous incidence of joint lesions in this strain to be 4%.

Description of joint lesions

A continuous spectrum of synovitis was observed which was arbitrarily divided into mild, moderate and severe categories (Table 2).

'Mild' lesions were characterised by light focal subsynovial infiltration by polymorphs, lymphocytes, and occasional plasma cells. The synovial lining layer remained flat in many cases, while in others mild cuboidal hyperplasia was evident (Fig. 3b).

'Moderate' lesions were characterised by a denser and more confluent inflammatory infiltrate containing greater numbers of lymphocytes and plasma cells. Synovial cells showed cuboidal hyperplasia and a degree of multilayering. Mild increases in vascularity were sometimes apparent. Inflammatory villous configuration started to become a feature.

'Severe' lesions were characterised by a dense inflammatory infiltrate, with the formation of a small germinal centre in one instance. Inflammatory villus formation was conspicuous as was multilayering of the synovial cells. In some instances fibrin was incorporated into the synovial layer and vascularity

Table 1 Incidence of synovial lesions in rabbits injected intravenously with killed bacteria

<table>
<thead>
<tr>
<th>Strain of rabbit</th>
<th>Killed bacteria (injected IV)†</th>
<th>Incidence of joint lesions (%)</th>
<th>Incidence of moderate to severe lesions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Old English'</td>
<td>E.coli</td>
<td>71 (5/7)*</td>
<td>43 (3/7)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0 (0/7)</td>
<td>0 (0/7)</td>
</tr>
<tr>
<td>'Sandy Lop'</td>
<td>E.coli</td>
<td>88 (7/8)</td>
<td>0 (0/8)</td>
</tr>
<tr>
<td></td>
<td>B.subtilis</td>
<td>38 (3/8)</td>
<td>0 (0/8)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14 (1/7)</td>
<td>0 (0/7)</td>
</tr>
</tbody>
</table>

* Actual number of rabbits used is shown in parentheses.
† IV=intravenously.
54 Hanglow, Welsh, Conn, Pitts, Rampling, Coombs

Table 2 Histological assessment of synovial lesions in rabbits injected intravenously with killed bacteria

<table>
<thead>
<tr>
<th>Strain of rabbit (injected IV)</th>
<th>Bacteria</th>
<th>Rabbit No and sex</th>
<th>Overall classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Old English' 5/7 injected</td>
<td>E.coli</td>
<td>6510 ♀</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6511 ♂</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6512 ♂</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6519 ♂</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6620 ♀</td>
<td>+</td>
</tr>
<tr>
<td>'Sandy Lop' 7/8 injected</td>
<td>E.coli</td>
<td>6561 ♂</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6562 ♂</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6563 ♂</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6564 ♀</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6565 ♂</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6566 ♂</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6571 ♂</td>
<td>+</td>
</tr>
<tr>
<td>B.subtilis 3/8 injected</td>
<td></td>
<td>6572 ♂</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6575 ♂</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6579 ♂</td>
<td>+</td>
</tr>
<tr>
<td>Control 1/7</td>
<td></td>
<td>6558 ♀</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neutrophils</th>
<th>Plasma cells</th>
<th>Lymphoid cells</th>
<th>Germinal centres</th>
<th>Fibrosis</th>
<th>Cuboidal/flat synovial lining cells</th>
<th>Villous</th>
<th>Fibria</th>
<th>Number of layers of synovial lining cells</th>
<th>Unilateral or bilateral lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>U</td>
</tr>
<tr>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>C</td>
<td>+++</td>
<td>-</td>
<td>4</td>
<td>B</td>
</tr>
<tr>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>C</td>
<td>+++</td>
<td>+</td>
<td>3</td>
<td>B</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>F/C</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>U</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>F/C</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>U</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>F/C</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+/−</td>
<td>-</td>
<td>F/C</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>U</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>F/C</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>U</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>B</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>U</td>
</tr>
</tbody>
</table>

The interpatellar, infrapatellar, and lateral synovia were dissected from both knees of each rabbit after death, fixed in formalin, and stained with haematoxylin and eosin as described in the text. Severity of joint lesions is assessed relative to normal rabbit synovia [-]. Joint lesions are scored as either mild [+], moderate [++], or severe [+++]. A similar coding for infiltration by neutrophils, plasma cells, and lymphocytes has been used. Synovial lining cells are classified as either cuboidal (C), flat (F), or focally cuboidal (F/C).

was greatly increased (Figs 3c and 3d). In one animal inflammation spread into the adjacent fibrocartilage which showed evidence of vascularisation (Fig. 3e). Mild lesions were usually unilateral (only one joint affected), while moderate to severe lesions affected both knees to a similar degree.
Induction of rheumatoid factor and joint lesions in rabbits

Fig. 3c

Fig. 3d

Fig. 3e

Fig. 3 Histological classification of joint lesions. (a) Normal synovium from a control rabbit. A single layer of flat synovial cells is present on the surface. Beneath lies unremarkable adipose tissue. (Haematoxylin and eosin, ×425). (b) An area of lightly inflamed synovium from a 'Sandy Lop' rabbit injected with E.coli. Synovial cells have an increased amount of cytoplasm and lie two to three cells deep. The underlying chronic inflammatory cells are lymphocytes and plasma cells in approximately equal proportions. Capillary blood vessels are prominent, suggesting increased vascularity. (Haematoxylin and eosin, ×300). (c) Heavily inflamed synovium from an 'Old English' rabbit injected with E.coli. Synovial cells have a considerably increased amount of cytoplasm and lie four to five cells deep on the surface. There is a dense chronic inflammatory cell infiltrate immediately beneath the hyperplastic synovial cells. (Haematoxylin and eosin, ×300). (d) Adjacent inflammatory villi from an 'Old English' rabbit injected with E.coli. This was classed as severe inflammation. Synovial cells lie two to three deep and have abundant cytoplasm; occasional binucleate forms are present. The cores of the villi are rich in plasma cells and, at higher magnification, appear rather vascular. (Haematoxylin and eosin, ×120). (e) Fibrocartilage from the severely inflamed joint of an 'Old English' rabbit injected with E.coli. At the top of the figure (slightly crushed) lies part of the inflammatory infiltrate of lymphocytes and plasma cells. A few of these cells extend more deeply into the fibrocartilage and are associated with tiny capillaries which have plump endothelial cells (arrows), the appearance being those of inflammatory neovascularisation. (Haematoxylin and eosin, ×375).
Lesions in 3/7 'Old English' rabbits were classified as moderate to severe and in 2/7 as mild.

In contrast, all the lesions observed in the 'Sandy Lop' rabbits injected with either killed *E. coli* or *B. subtilis* were mild. There was no detectable qualitative difference between the nature of the lesions in 'Old English' and 'Sandy Lop' rabbits.

Kidneys were available for study from all but one animal. In no case was there evidence of glomerulonephritis.

**Analysis of cells recovered from synovial fluids**

**Absolute numbers of nucleated cells**

'Old English'. The mean number of viable nucleated cells recovered after death from the synovial fluids of untreated 'Old English' rabbits was 8240 (range 6000–15 360) (Fig. 4). Increased numbers were recovered from those rabbits injected with *E. coli* that developed joint lesions (mean 304 600, range 16 300–816 000).

'Sandy Lop'. The mean number of nucleated cells recovered from the synovial fluids of 'Sandy Lop' rabbits, injected with either *E. coli* or *B. subtilis* did not exceed 25 000. There was no significant increase over control values, which ranged from 1000 to 25 000 (mean 10 700) (data not shown).

**T cells levels in synovial fluids**

The mean absolute number of T cells recovered from the synovial fluids of untreated 'Old English' rabbits was 370 (range 100–1075). These values reflect a mean of 4% of total nucleated cells (range 1–7%). *E. coli* injected rabbits with joint lesions had increased absolute numbers and percentage of T cells in the synovial fluids, i.e., mean value of 77 670 (range 2610–179 520). The percentage of T cells within the synovial fluid ranged between 1 and 60% (mean 23%) (Table 3).

**High titres of serum RF were not associated with the development of joint lesions**

Although the highest levels of serum RF were

---

**Table 3 Absolute number of T cells recovered from the synovial fluids of 'Old English' rabbits injected with killed E. coli**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rabbit number and sex</th>
<th>Knee joint</th>
<th>Severity of joint lesion</th>
<th>T cells (%)</th>
<th>Absolute number of T cells recovered from synovial fluid</th>
<th>Mean value±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> injected with joint lesions</td>
<td>6510 ♀</td>
<td>R</td>
<td>+</td>
<td>5</td>
<td>8 480</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6511 ♂</td>
<td>R</td>
<td>+++</td>
<td>60</td>
<td>165 600</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6512 ♀</td>
<td>L</td>
<td>+++</td>
<td>41</td>
<td>132 600</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6519 ♂</td>
<td>R</td>
<td>+++</td>
<td>22</td>
<td>179 520</td>
<td>77 670±74 520</td>
</tr>
<tr>
<td></td>
<td>6620 ♂</td>
<td>L</td>
<td>+</td>
<td>16</td>
<td>2 610</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> injected without joint lesions</td>
<td>6509 ♀</td>
<td>R</td>
<td>–</td>
<td>72</td>
<td>1 460</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6617 ♂</td>
<td>L</td>
<td>–</td>
<td>17</td>
<td>1 500</td>
<td>1750±500</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>5633 ♀</td>
<td>R</td>
<td>–</td>
<td>7</td>
<td>730</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5635 ♂</td>
<td>L</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5638 ♀</td>
<td>R</td>
<td>–</td>
<td>3</td>
<td>1 075</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5641 ♂</td>
<td>L</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>370±340</td>
</tr>
<tr>
<td></td>
<td>6618 ♂</td>
<td>L</td>
<td>–</td>
<td>7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6621 ♂</td>
<td>L</td>
<td>–</td>
<td>4</td>
<td>&lt;100</td>
<td></td>
</tr>
</tbody>
</table>

T cells in synovial fluids (from both right [R] and left [L] knee joints) were identified by their ability to form rosettes with papain-treated rabbit erythrocytes. Severity of joint lesions was assessed relative to normal rabbit synovia [–]. Joint lesions are scored as either mild [+], moderate [++] or severe [+++].
Induction of rheumatoid factor and joint lesions in rabbits

Discussion

Repeated intravenous injections of killed bacteria stimulate the production of IgM RF and rheumatoid-like synovial lesions in rabbits. These RFs are true autoantibodies reacting with IgG from the same rabbit. Several other groups have described the development of rheumatoid factor-like substances (RFLS) in rabbits after stimulation with protein,11 bacterial12 or parasitic13 antigens and after injections of ammonium sulphate precipitated autologous rabbit IgG.14 In all these systems RFLS were measured by their reactivity to either heat-aggregated human IgG or pooled heat-aggregated rabbit IgG. Therefore, they neither necessarily measure true autoantibodies to autologous IgG nor eliminate antiallotypic antibodies. However, joint lesions were not described in any of these other reports; joint inflammation could easily have been overlooked without histological examination. In our experimental rabbits with moderate to severe lesions no impairment of movement or gross joint swelling was observed.

In addition, we have observed that injections of killed B.subtilis induced higher levels of RF than did E.coli (Fig. 1). This is in contrast to the observations of Abruzzo and Christian1 who found B.subtilis to be a poor stimulator of RF compared with E.coli. However, this discrepancy is probably attributable to the differences in assay systems employed for measuring RF and differences in breeds of rabbits used. High levels of IgM RF were associated with high titres of IgG directed against the injected bacteria (Fig. 2). This is consistent with Abruzzo and Christian’s hypothesis that RF is directed against specific IgG present in immune complexes containing bacterial antigens.

The mechanisms resulting in the development of synovitis in rabbits injected repeatedly with killed bacteria are not understood. The development of chronic erosive polyarthritis in rats injected intra-peritoneally with group A streptococcal cell walls is thought to depend upon the deposition and persistence of bacterial cell walls in synovial tissues.15 If a similar mechanism were operating in the present study we would have expected a similar incidence of joint lesions in the ‘Sandy Lop’ rabbits injected with either E.coli or B.subtilis and this was not the case.

It is possible that the RF itself may be initiating or contributing to the development of joint lesions. Reactivity against self IgG has been postulated to be an important mechanism initiating joint inflammation in human rheumatoid arthritis.16 However, we found that although B.subtilis injected rabbits developed the highest titres of serum RF, only 3/8 animals developed mild synovitis. In contrast, mod-

![Graph](image-url)

**Fig. 4** Correlation between the severity of joint lesions and total nucleated cells recovered from the synovial fluids of ‘Old English’ rabbits injected with killed E.coli. The total number of nucleated cells recovered from the synovial fluids of E.coli injected (×) and control (●) animals has been plotted against the severity of joint lesions. Joint histology has been classified as either normal (–), mild lesions (+), moderate lesions (++), or severe lesions (+++).

detected in ‘Sandy Lop’ rabbits injected with killed B.subtilis, only 3/8 of these rabbits developed joint lesions, all of which were classified as mild. Indeed, the rabbit with the highest titre (256) did not show any joint inflammation. In contrast, the most severe joint changes were seen in the ‘Old English’ rabbits injected with killed E.coli which did not show high titres of serum IgM RF (Fig. 5).
erate to severe lesions were seen in the 'Old English' rabbits injected with *E. coli* which had the lowest titres of RF. This result may be a consequence of the strain differences, since 'Old English' rabbits show increased susceptibility to experimentally induced arthritis, e.g., serum sickness and the Glynn-Dumonde model of arthritis (Hunneyball, personal communication). It is also possible that the high titres of RF detected in *B. subtilis* injected rabbits have a protective effect. RF production may be a normal physiological process involved in the clearance of immune complexes and elimination of antigen, as is the production of immunoconglutinin, i.e., antibody to iC3b. Further evidence to support this hypothesis comes from the work of Nemazee and Sato who showed that the 129/Sv mouse strain produces a very high RF-like antibody in response to an unknown environmental stimulus in the absence of obvious disease.

Alternatively, high serum levels of IgM RF may not be associated with severe joint lesions, because serum RF is not representative of RF produced elsewhere in the tissues including synovium. Any RF produced in the synovium and highly avid for IgG may never reach the blood in detectable amounts; instead it may become trapped in the tissue or vessel walls of the synovium as immune complexes are locally precipitated. This could result in the direct initiation of inflammation. RF bound to immune complexes could also be removed by the reticuloendothelial system and therefore not be available for measurement. As yet, for technical reasons, we have only measured serum IgM RF.

Abruzzo and Christian, whose protocol for RF induction we have followed, claimed to have measured an IgG RFLS. IgG anti-IgG may make a more direct contribution to the development of arthritis. This study is, to the best of our knowledge, the first report of experimentally induced arthritis in rabbits concurrent with true RF production measured by reactivity with self-IgG, without the use of Freund's complete adjuvant or injection of antigen directly into the joint. The synovial lesions are rheumatoid like with the synovium infiltrated by mixed chronic inflammatory cells. Germinal centre and villi formation were also observed in the most

Fig. 5 Lack of correlation between high IgM RF titres and joint lesions in rabbits injected with killed bacteria. Serum IgM RF titres in (a) 'Old English' rabbits injected with *E. coli*; (b) 'Sandy Lop' rabbits injected with *E. coli*, and (c) 'Sandy Lop' rabbits injected with *B. subtilis*. Joint histology in control (○) and injected (×) animals has been classified as either normal (−), mild lesions (+), moderate lesions (++), or severe lesions (+++).
severe lesions, and T cells were present in increased number in the synovial fluids of affected rabbits (Table 3). Glomerulonephritis was not observed in any of these animals.

*E. coli* has been implicated in human arthritis development as a consequence of intestinal bypass operations for morbid obesity.²³ It is a member of the *Enterobacteriaceae* which includes yersinia, shigella, salmonella, and klebsiella species, organisms implicated in HLA-B27 associated reactive arthritis and ankylosing spondylitis, though reactive arthritis are not associated with IgM RF production.²⁴ Further studies of this bacterially induced model of arthritis may give an understanding to the development of joint lesions after minimal but repeated bacteraemic episodes which could arise (amongst other situations) through an alteration in gut flora, perhaps as a consequence of dietary changes. For example, erosive joint lesions have been described in piglets fed on a protein rich diet.²⁵ This arthritis was associated with the development of an abnormal intestinal flora with high levels of *Clostridium perfringens* type A which produce alpha toxins, increasing gut permeability.

We would like to acknowledge Miss Louise Forsdyke and Ms Beverley Wilson for excellent technical assistance, and Mrs Anita Hancock who assisted in the preparation of this manuscript. This work was supported by the Arthritis and Rheumatism Council, UK.

References

7 Kelus A S, Gell P G H. Immunoglobulin allotypes of experimental animals *Prog Allergy* 1967; 11: 141.
Experimental induction of rheumatoid factor and joint lesions in rabbits after intravenous injections of killed bacteria.

A C Hanglow, C J Welsh, P Conn, J M Pitts, A Rampling and R R Coombs

Ann Rheum Dis 1986 45: 50-59
doi: 10.1136/ard.45.1.50

Updated information and services can be found at:
http://ard.bmj.com/content/45/1/50

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.

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/