BACTERIA-SPECIFIC LYMPHOCYTE PROLIFERATION IN PERIPHERAL BLOOD AND RELATED DISEASES

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SUMMARY

The cellular immune response seems to be important for the pathogenesis of reactive arthritis (ReA) and a bacteria-specific lymphocyte proliferation (LP) is often found in synovial fluid (SF) of ReA patients. However, the role of the bacteria-specific LP in peripheral blood (PB) is less well defined. In this study, we investigated 215 paired samples of SF and PB from patients with ReA (n = 65), undifferentiated oligoarthritis (n = 133) and undifferentiated spondylarthropathy (n = 17) to analyse the LP in PB and SF in relation to time. In 24 out of 87 patients (27.6%) with a bacteria-specific LP in synovial fluid, a positive LP to the same bacterium was also found in PB. While a positive LP in SF was found most frequently in the first week of the arthritis, a positive LP in PB was detected in 45% of patients when investigated between weeks 2 and 4 after the onset of arthritis, but was rarely found very early and late in the course of the arthritis. The time point seems to be crucial for the investigation of an LP in PB in patients with ReA.

KEY WORDS: Cellular immune response, Peripheral blood, Reactive arthritis.

REACTIVE arthritis (ReA) occurs after a preceding infection of the urogenital tract with Chlamydia trachomatis or of the gastroenteral tract with Yersinia, Salmonella, Shigella and other bacteria. Recently, we could show that Chlamydia pneumoniae can also induce ReA, although this microbe seems to be less important [1]. ReA-associated bacteria have been detected in the joint by various techniques [2–6], indicating local persistence of bacterial antigen.

The concept that these antigens drive the local immune response is supported by the finding of an antigen-specific lymphocyte proliferation (LP) to the triggering bacterium [7–9] which is nearly always much higher in synovial fluid (SF) compared to peripheral blood (PB). Furthermore, the antigen-specific T-cell frequency determined by limiting dilution was also reported to be higher in SF than in PB [10]. Therefore, the LP is rarely used in PB, mainly because in the majority of patients this test is negative.

Several studies claim that the antigen-specific LP in PB may even be lower in patients with acute ReA, compared either with the same patients in remission [11] or with patients infected with one of the ReA-associated bacteria but not developing ReA [12, 13]. Similar differences between SF and PB have been reported for the cellular immune response to Klebsiella pneumoniae in patients with ankylosing spondylitis [14].

We have investigated paired samples of PB and SF in 215 patients with ReA, undifferentiated oligoarthritis (UOA) and undifferentiated spondylarthropathies (uSpA) in order to clarify the relevance of the LP in PB cells in these arthritides. The latter two groups have a clinical picture suggestive of ReA, but without a clear diagnosis of ReA. About one-third of patients with an antigen-specific LP in SF also showed a cellular immune response to the same bacterium in PB. The highest proportion of an antigen-specific LP in PB cells was found between the second and the fourth week after the first symptoms of ReA.

PATIENTS AND METHODS

Patients

Patients were recruited from four rheumatology clinics in the Berlin area. Paired samples of PB and SF were obtained from 215 patients with either ReA (n = 65), UOA (n = 133) or uSpA (n = 17). Further demographic characteristics are shown in Table I. The synovial lymphocyte proliferative responses of some of the patients have been reported before [10, 15, 16]. A puncture of a vein or joint was only performed when necessary for diagnostic or therapeutic reasons. All patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs) and some of the patients also occasionally with intra-articular glucocorticoid injections and/or sulphasalazine.

Definitions

Reactive arthritis was defined as oligoarthritis preceded by a clear history of urethritis [urogenital (u) ReA] or gastroenteritis [enteral (e) ReA] in the previous 4 weeks. The triggering bacterium was identified either by a positive culture from stool or urethral swab, or by a significantly elevated antibody titre early in arthritis [17]. UOA was defined as arthritis of unknown cause involving ≤4 joints including the knee, with no history of a preceding infection or extra-articular features suggestive of Reiter’s syndrome. Other rheumatic diseases were excluded by appropriate investigations. Undifferentiated spondylarthropathy was defined as

Submitted 19 May 1997; revised version accepted 5 November 1997.

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inflammatory back pain and/or oligoarthritis predominantly of the lower limbs together with one of the following features: enthesitis, alternating buttock pain, or a positive family history according to the European Spondylarthropathy Study Group criteria. Patients with defined spondylarthropathies except those with ReA were not included in this study.

Cell separation and culture

Mononuclear cells (MNC) from PB and SF were separated as described previously [15], and triplicate wells were stimulated with the following agents in 87 patients when this study was started: tissue culture medium alone (background proliferation); *Chlamydia trachomatis* serovar L1 (5 μg/ml) *Yersinia enterocolitica* O.3 and O.9 (3 μg/ml); tetanus toxoid (Behring, Marburg, Germany; 1 μg/ml); or pokeweed mitogen (Sigma, Poole; 1 μg/ml) [10, 15, 16]. In the remaining 128 patients, the following were used additionally as antigens: *Salmonella enteritidis* (5 μg/ml), *Shigella flexneri* (5 μg/ml) and *Campylobacter jejuni* (5 μg/ml). All bacteria were heat inactivated at 60°C for 1 h.

Wells were pulsed with $[^{3}H]$thymidine (7.4 kBq/well) for the last 18 h of culture and incorporation measured at day 6 as described previously [15]. Stimulation was carried out with whole bacteria.

Results are expressed as $[^{3}H]$thymidine incorporation in counts per minute (c.p.m.) or in stimulation indices (SI). Stimulation indices, defined as the proliferation induced by an antigen divided by the background proliferation, were also used to define the positivity and specificity of responses as follows: in SF, SI ≥ 5 were considered positive. If responses to two or more antigens were positive, the highest SI had to be double the value of the next highest to be considered specific, otherwise this was regarded as a non-specific response. Subjects with SI < 5 for all pathogens were considered to be non-responders [15]. In PB, an SI ≥ 3 was regarded as positive. It was necessary to determine a different level for positivity in the two compartments since LP to specific and non-specific antigens is always much higher in SF. This might be due to the higher number of antigen-specific T cells in SF of ReA patients or to highly activated T cells and macrophages found in SF compared to PB (for further discussion, see refs 10 and 19).

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**TABLE I**

<table>
<thead>
<tr>
<th>Characteristics of patients with ReA (reactive arthritis), UOA (undifferentiated oligoarthritis) and uSpA (undifferentiated spondylarthropathy)</th>
<th>All patients</th>
<th>ReA*</th>
<th>UOA</th>
<th>uSpA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>215</td>
<td>65</td>
<td>133</td>
<td>17</td>
</tr>
<tr>
<td>HLA B27 (%)</td>
<td>37</td>
<td>71</td>
<td>19</td>
<td>86</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>129/86</td>
<td>47/18</td>
<td>69/64</td>
<td>13/4</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>36.4</td>
<td>33.5</td>
<td>37.5</td>
<td>39.3</td>
</tr>
<tr>
<td>(Range)</td>
<td>(11–84)</td>
<td>(16–82)</td>
<td>(11–84)</td>
<td>(17–75)</td>
</tr>
<tr>
<td>Median disease duration (weeks)</td>
<td>20.0</td>
<td>6.5</td>
<td>34.5</td>
<td>16.0</td>
</tr>
<tr>
<td>(Range)</td>
<td>(1–1225)</td>
<td>(1–555)</td>
<td>(1–1025)</td>
<td>(2–1225)</td>
</tr>
</tbody>
</table>

*All uSpA patients had a knee effusion, seven an enthesitis, four a positive family history (as defined in the ESSG criteria [18]) and six an enthesitis plus a positive family history.

†All ReA patients had a positive family history according to the European Spondylarthropathy Study Group criteria [18].

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Statistical analysis

Results were analysed using the $\chi^2$ test and Fisher’s exact test for non-parametric data.

RESULTS

Bacterium-specific lymphocyte proliferation in peripheral blood compared to synovial fluid

Eighty-seven patients out of 215 showed a specific proliferation to an ReA-associated bacterium in SF. In 24 out of these 87 patients (27.6%), an LP to the same bacterium was also found in PB (Fig. 1). Only two patients proliferated to a different bacterium in PB compared to SF (data not shown). The highest proportion of a positive LP in PB could be detected in patients with uSpA followed by patients with ReA and UOA (Fig. 1). The percentage of patients positive for one of the bacteria in their LP from SF differed between the groups: *Yersinia* was most frequent for UOA patients, while in ReA and uSpA *Chlamydia* was found most often. In PB, *Chlamydia* were the predominant bacteria detected by LP for all three patient groups (Fig. 2). Interestingly, the percentage of *Shigella*-specific LP in PB was higher with 56% (five out of nine patients) and the *Yersinia*-specific LP was lower with 11% (four out of 38 patients) in comparison to 35% in the *Chlamydia* group (14 out of 40 patients).

FIG. 1.—Percentage of specific lymphocyte proliferation (as defined in Patients and methods) in peripheral blood to reactive arthritis-associated bacteria in all patients, patients with reactive arthritis (ReA), undifferentiated oligoarthritis (UOA), and undifferentiated spondylarthropathies (uSpA), who showed a specific lymphocyte proliferation to the same bacterium in synovial fluid.
Bacterium-specific lymphocyte proliferation at different time points

The comparison of bacterium-specific LP in SF and PB became even more interesting when the time elapsed since the first symptoms of arthritis was analysed. Only a slight non-significant decrease in the percentage of bacterium-specific LP was found in SF over the first half year of arthritis, with a peak in the first week. However, the maximum frequency of positive antigen-specific LP in PB was found when patients were investigated between 2 and 4 weeks after the onset of arthritis. This was significantly higher compared to the time after week 26 (P < 0.01) and non-significantly (most likely due to the small numbers) higher compared to the first week (P > 0.05) and the time after the fourth week (P > 0.05) (Fig. 3). This difference was even clearer when the percentage of positive LP in PB was calculated only for those patients who showed an LP to the same bacterial antigen in SF: 45% (17/37) of patients were positive between weeks 2 and 4, 29% (16/59) were positive between weeks 5 and 26, but only 12% (1/9) and 11% (13/108) were positive in the first week and after week 26, respectively (Fig. 3; P < 0.05 for the comparison between weeks 2 and 4 and after week 26).

Serial investigations of bacterium-specific lymphocyte proliferation in peripheral blood

In 12 out of the 24 patients who showed a positive LP in PB and SF, an LP was performed in PB more than once. Only three patients (patients 2, 3 and 5) showed a specific LP to the same bacterium each time they were tested (Fig. 4): patient number 2 in weeks 2, 3 and 15, patient number 3 in weeks 4 and 5, and patient number 5 in weeks 8 and 36. These results support the finding that a positive bacterium-specific LP in PB is more likely to occur earlier in the course of arthritis except in the first week. Most of these patients were followed up for at least 1 yr. There was no correlation between the results of the bacterium-specific LP in PB and the occurrence of remission or chronicity in the course of the patients’ arthritis.

DISCUSSION

In this study, we showed that nearly 30% of 87 patients with a bacterium-specific LP in SF also had an LP to the same bacterium in PB. This figure comes close to that reported by Ford et al. [20] in a much smaller study in which they detected such a systemic response in four out of 12 patients (33%). The proportion of positive LP in PB was different for C. trachomatis (35.1%), Y. enterocolitica (11.4%) and S. flexneri (55.6%) in our study. The reason for this is not clear. That patients with *Shigella*-induced arthritis were investigated more frequently earlier in the course of their arthritis compared to the other bacteria might be one explanation.

We then analysed the cellular immune response in relation to the duration of the arthritis. The highest frequency of a positive LP in SF was found in the first week. In contrast, nearly all of the LP to the same bacterium were negative in PB at the time of the first week, while there was a clear peak of positive LPs in PB during the 2nd to 4th weeks. These results are of interest for two reasons. (i) They suggest that a systemic response occurs at best only transiently during the initial gut or urogenital tract infection, and that the cellular immune response starts locally after deposition of bacterial antigen in the joint. The systemic cellular immune response in arthritis appears later and is not detectable before the second week. The faster decrease in the cellular immune response in PB com-
pared to SF over the following weeks also argues for a predominant local cellular response. (ii) The bacterium-specific LP in PB is of no use for diagnostic purposes during the first week of the arthritis or after the 26th week, while the best results—up to nearly 50% positivity—can be achieved after the first week.

The low systemic immune response to ReA-associated bacteria reported before suggests that this immune response is ineffective, and thus might fail to prevent spreading of bacteria to the joint and the occurrence of arthritis [11–14, 21]. The data reported here give some support to this concept.

Based on these considerations, it would have been expected that patients with a positive LP in PB were more likely to go into remission. Although we could not find such a correlation, the positive LP observed in our study might not have been strong enough for fighting the bacteria effectively.

In our study, PB data were analysed which showed an antigen-specific LP in their SF. However, the separation of synovial lymphocytes is labour intensive, and the sensitivity and specificity of the antigen-specific synovial LP have not been determined. Therefore, such a study would be performed easier in the future in ReA patients in whom bacteria can be detected in their joints by polymerase chain reaction (PCR). This is especially true for C. trachomatis [22], whereas the diagnostic value of bacteria-specific PCR is less clear for the enterobacteria [23, 24].

In summary, our data suggest that the role of systemic cellular immune responses to the triggering bacteria might have been underestimated in the past because blood was not investigated at the optimal time. Future studies comparing the antigen-specific cellular immune response between PB and SF should pay more attention to the time point of investigation. Although the LP of PB MNC will not play a role in the diagnostic procedure in ReA because of a low sensitivity and an uncertain specificity, it might be useful for investigative purposes [23–25].

REFERENCES
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