Muramyl Dipeptide-Induced Adjuvant Arthritis

SHIGEKI NAGAO AND ATSUSHI TANAKA*

Department of Biochemistry, Shimane Medical University, Iwami 693, Japan

Muramyl dipeptide, N-acetylmuramyl-L-alanyl-D-isoglutamine, induced adjuvant arthritis in WKA rats when injected in a water-in-oil emulsion prepared with Freund incomplete adjuvant (Difco), but not when emulsified with Drackeol and Arlacel A.

In 1956, a single intracutaneous injection of heat-killed tubercle bacilli incorporated in a water-in-oil emulsion was reported to induce an arthritis in rats which is now called adjuvant arthritis (15). Since then, mycobacterial wax D, bacterial cell walls of different species, their peptidoglycans, and fragments of the peptidoglycans were all found to be arthritogenic (2, 6, 8, 9, 14). Recently, among the peptidoglycan fragments tested, disaccharide-heptapeptide-disaccharide (N-acetylglycosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanyl-meso-diaminopimelyl-D-isoglutaminyl-L-alanyl-N-acetylmuramyl-N-acetylglycosamine) was found to be the smallest arthritogenic structure (7, 12). Structures smaller than this, for instance, synthetic muramyl dipeptide (MDP, N-acetylmuramyl-L-alanyl-D-isoglutamine), and products of enzymatic cleavage of glycan chains (-GlcNAc-MurNAc-) of peptidoglycans were not arthritogenic, though both MDP and the enzymic cleavage products were still active as adjuvants (6, 7, 11). For these reasons, it was thought that two or more disaccharide units were needed for the induction of adjuvant arthritis and the minimal structure necessary for adjuvant activity was not sufficient.

The present study shows that synthetic MDP can induce severe arthritis in rats. Female WKA rats, 10 weeks of age, from the Institute for Experimental Animals, Kyushu University, Fukuoka, Japan, were injected intracutaneously into back hind footpads with 0.1 ml of a water-in-oil emulsion containing 0.1 mg of MDP. To prepare the emulsion, we emulsified a phosphate-buffered saline (PBS), pH 7.2, containing MDP at a concentration of 2 mg/ml with an equal volume of Freund incomplete adjuvant (Difco Laboratories, Detroit, Mich., lot no. 636671 and 640223) using a sonicator (Sonifier, model W-200P, Branson Instruments Co., Stamford, Conn.). Animals were observed daily for 4 weeks after injection, and the degree of developed lesions and the days of onset were evaluated as described previously (26).

Arthritis occurred in about 50% of the rats injected with MDP (Table 1). Some of them showed particularly severe arthritis (Fig. 1). The ankle, tarsal, metatarsal, and phalangeal joints of all the legs were involved, and the tails were beaded. The day of onset was between 16 and 21 days. Rats injected with the emulsions containing no MDP or MDP with ovalbumin (100 µg) failed to develop arthritis. The site of MDP injection of rats that developed arthritis showed a marked swelling that became manifest about 1 week after injection. A correlation between the swelling and the development of polyarthritis was statistically significant (correlation coefficient, 0.9; P < 0.005). However, no correlation appeared to exist between the development of adjuvant arthritis and the weight of draining lymph nodes, which was shown to reflect the extent of granuloma formation by MDP (5).

Rats developing adjuvant arthritis showed negative skin reactions when skin was tested with MDP (100 µg) 3 weeks after the injection.

We reported previously that MDP was active as an adjuvant in WKA rats (21). Though it has been suggested that the structure of MDP may not be sufficient for the induction of adjuvant arthritis (7, 12), the present study clearly shows that MDP did induce adjuvant arthritis. This finding may be of importance because of the following two points. First, it suggests that any attempts to use MDP with humans should be done cautiously. It was reported that 6% of the patients who had received Mycobacterium bovis (BCG) immunotherapy developed an arthritis which was thought to be adjuvant arthritis (22).

Second, it may contribute to our understanding of the pathogenesis of adjuvant arthritis. It appears certain that adjuvant arthritis develops as a result of some immunological response, probably of the delayed type (16, 25). However, responsible antigens are still obscure (3, 17, 19, 23, 25). No definite immunogenicity has been so far detected in MDP (1, 5). This was confirmed in the present study; MDP did not sensitize rats against MDP (Table 1). Therefore, although the question of whether MDP in some way can
TABLE 1. Induction of adjuvant arthritis by MDP

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Inoculum</th>
<th>Incidence</th>
<th>Skin reaction at 24 h*</th>
<th>Hind footpad thickness (mm; mean ± SD)</th>
<th>Wt of lymph node (mg; mean ± SD)</th>
<th>Arthritis score</th>
<th>Onset (days)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>None MDP</td>
<td>0/5 ND</td>
<td>4.9 ± 0.4 4.3 ± 0.4 ND</td>
<td>0</td>
<td>18-21</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td>3/7 ND</td>
<td>5.0 ± 0.0 5.0 ± 0.0</td>
<td>5.0</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDP + ovalbum</td>
<td>0/4</td>
<td>6.5 ± 0.9 4.9 ± 0.3</td>
<td>7.4 ± 0.2 5.1 ± 1.1</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>None MDP</td>
<td>0/5 2.0 ± 0.1</td>
<td>4.7 ± 0.4 4.3 ± 0.2</td>
<td>77 ± 13</td>
<td>0</td>
<td>16-18</td>
<td></td>
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<tr>
<td></td>
<td>MDP</td>
<td>3/5 2.1 ± 0.1</td>
<td>5.5 ± 0.0 4.0 ± 0.0</td>
<td>93</td>
<td>8</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDP + ovalbum</td>
<td>0/4</td>
<td>7.8 ± 0.0 5.9 ± 2.1</td>
<td>115 ± 26</td>
<td>13</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Rats were injected with each inoculum into the left hind footpads. Freund incomplete adjuvant (Difco lot no. 636671) was used to prepare the inocula. The amount of MDP or ovalbumin was 100 μg. Three weeks later, the rats were skin tested either with 100 μg of MDP (control and MDP groups) or with 100 μg of ovalbumin (MDP + ovalbumin group), the thickness of the hind footpads was measured, the degree of polyarthritis was scored, and the weight of draining lymph nodes was weighed after sacrifice. For the MDP groups, the values of measurements of each rat were also shown. ND, Not determined; SD, standard deviation.

Double skin thickness was measured. Numbers indicate mean thickness ± standard deviation in millimeters.

activities of MDP other than antigenicity (5, 13, 18, 20). Also, the possibility that MDP may induce virus infection cannot be excluded (4).

It is well known that the development of adjuvant arthritis is dependent on oil vehicles in which arthritogenic substances are injected (10, 24). This was confirmed in the present study (Table 2). Adjuvant arthritis was induced only when MDP-containing PBS was emulsified with an equal volume of Freund incomplete adjuvant (Difco) (Arlacel A-Bayol F, 8.5:1.5). A similar Freund-type emulsion prepared by mixing MDP-containing PBS with an equal volume of the mixture of Arlacel A (Atlas Powder Co., Wilmington, Del.) and Drackol 6-VR (Penreco, Los Angeles, Calif.) (8.5:1.5) did not cause adjuvant arthritis (Table 2). This result contrasts with granuloma formation by MDP; both of the

TABLE 2. Effect of oil vehicle on the induction of adjuvant arthritis by MDP

<table>
<thead>
<tr>
<th>Oil vehicle</th>
<th>Inoculum</th>
<th>Incidence</th>
<th>Mean score</th>
<th>Onset (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDP (100 μg)</td>
<td>0/26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>0/4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MDP (100 μg)</td>
<td>5/6</td>
<td>10</td>
<td>18-25</td>
<td></td>
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</table>

* Rats were injected with inocula into left hind footpads. Freund incomplete adjuvant (Difco lot no. 640223 and 640223) was used to prepare the inocula.

FIG. 1. (A) Diffuse swelling of both digits and carpal regions. (B) Swelling and nodulation of the tail.

become antigenic or even immunogenic in vivo still remains to be further studied, we are at the moment inclined to ascribe the mechanism of adjuvant arthritis induction not to the immunogenicity of MDP itself but to some self-anti-gen(s) generated by an action of MDP or to
above oil vehicles were effective to help MDP in granuloma formation (5). We cannot explain the reason why the use of the latter oil emulsion did not induce arthritis.

LITERATURE CITED


