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Adjuvant-Induced Arthritis in the Temporomandibular Joint of Rats

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When Lewis rats were immunized by intradermal injection into the parietal scalp rather than into the footpad with mycobacterial delipidated cells in squalene, arthritis could be produced in the temporomandibular joint (TMJ) with a maximum incidence of 60%, accompanied by systemic polyarthritis. Other methods of immunization including intradermal injection into the tail, posterior cervical region, or intra-inguinal lymph nodes did not induce arthritis in the TMJ. A combination of this inoculation and hemiocclusal loss markedly increased the incidence of arthritis in the TMJ. This arthritis in the TMJ was, however, milder than that in other joints and was apparent only histologically. The group given intradermal injection of adjuvants into the parietal scalp showed definite suppression of body weight gain. Since the method of intradermal injection into the parietal scalp can induce a high incidence of arthritis in the TMJ, our study presents a unique experimental model for study of arthritis in the TMJ.

In human rheumatoid arthritis, the incidence of temporomandibular joint (TMJ) involvement is 43 (3), 55 (5), or 71% clinically and 78.7% radiologically (1). We now have a number of animal models of systemic polyarthritis, such as adjuvant-induced arthritis (10), antigen-induced arthritis (4), collagen-induced arthritis (16) and alkyldiamine (CP-20,961)-induced arthritis (2). However, no one has ever demonstrated arthritis in the TMJ in these animal models. In preliminary studies, we failed to induce arthropathy in the TMJ in previously reported animal models. Newbould (9) first recommended that intra-lymph node injections were more reliable for production of experimental allergic encephalomyelitis than intradermal injections. Koga et al. (8) showed that intra-lymph node injections were the most potent method of producing adjuvant-induced arthritis. Even using this method, we failed to induce arthritis in the TMJ.

The lymph flow from the scalps of rats is to the submandibular lymph nodes. We have recently tried to inoculate *Mycobacterium* adjuvants into the scalp, with successful induction of arthritis in the TMJ. This report will present results about how to produce experimental arthritis in the TMJ and its incidence, clinical appearance, and histological features. We will also show that unilateral loss of occlusion, which itself does not produce arthritis in the TMJ, raises the incidence and severity of such adjuvant-induced arthritis.

MATERIALS AND METHODS

Animals. Wistar and Sprague-Dawley rats were obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan). Lewis rats were obtained from Charles River Breeding Laboratory (Wilmington, Mass.) and were maintained by mating siblings in the animal breeding center of Nagoya University, Nagoya, Japan. They were kept in groups of five or six in plastic cages and given water and standard rat chow ad lib. They weighed 140 to 200 g (female) or 220 to 290 g (male) and were 8 to 10 weeks of age at the time of immunization.

Adjuvants. Heat-killed, human mixed *Mycobacterium* strains (C, DT, and PN) were obtained from the Fisheries and Food Central Veterinary Laboratory (Weybridge, Surrey, England). These organisms were successively extracted with ether-ethanol (1:1) and chloroform and chloroform-methanol (2:1) according to the method of Asselineau (J. Asselineau, D.Sc. thesis, 1951). The delipidated cells thus obtained were triturated with a mortar and pestle and then mixed thoroughly with squalene (lot 107C-0020; Sigma Chemical Co., St. Louis, Mo.) or incomplete Freund adjuvant (Difco Laboratories, Detroit, Mich.).

Inoculation routes. The rats were injected intradermally only once with 0.1 ml of inocula containing 0.5 mg of *Mycobacterium* cells in the footpads, tails, posterior cervical region, or the parietal scalp. Both inguinal lymph nodes and both submandibular lymph nodes were also injected with 0.01 ml of inocula containing 0.5 mg of *Mycobacterium* cells.

Unilateral loss of occlusion. Amputation of the right incisors and extraction of the right molars of the rats were performed under ethyl ether anesthesia. Since the incisors were soon elongated, amputation was

performed every week. These treatments were conducted at various times in relation to the injection of adjuvants.

Visual observations. After inoculation, all rats were examined daily to evaluate the time of onset of polyarthritis and were graded from 0 to 4 for each appendage except for the injected one according to the method described in a previous paper (20). They were also weighed twice a week.

Histological studies. Since arthritis in the TMJ was not observed visually, all rats were sacrificed 5 weeks after immunization, and tissue specimens were fixed in 10% buffered formalin, decalcified by the method of Plank and Rychlo (12), and stained with hematoxylin and eosin. The scale for evaluation was: −, normal joint; ±, mild inflammatory changes; +, severe inflammatory changes; ++, severe inflammatory changes with bone destruction.

RESULTS

Failure in induction of arthritis in the TMJ via intra-inguinal lymph nodes injection. All rats were immunized in both inguinal lymph nodes with squalene-type or mineral oil-type (incomplete Freund adjuvant) adjuvant. Most (90%) of the Wistar rats and all of the Sprague-Dawley and Lewis rats developed systemic polyarthritis. The Lewis strain suffered from the severest disease, as reported previously (14, 19). Definite arthritis developed in both male and female rats injected with *Mycobacterium* cells mixed with squalene or incomplete Freund adjuvant. The clinical signs appeared about 9 to 12 days after immunization and gradually became more severe, finally reaching the maximum arthritogram score within a few days after appearance. Nevertheless, none of these rats was shown to have TMJ involvement in any of the histological examinations (Table 1).

Dissociated induction of arthritis in various joints with different routes of injection. It has been reported that induction of adjuvant-induced arthritis is highly successful with the following routes of injection: (i) footpad (intradermally) (10); (ii) intra-inguinal lymph nodes (8); (iii) tail (intradermally) (18); and (iv) posterior cervical region (interdermally) (11). In this study, we used two new injection routes: (v) intra-submandibular lymph nodes and (vi) parietal scalp (intradermally). To examine whether the joints involved differed depending on the routes of injection, we immunized rats, using various routes of injection. All rats were sacrificed for histological study 5 weeks after immunization, and the chief joints were examined in detail (Tables 2 and 3).

The most potent route for induction of systemic polyarthritis was the intra-lymph node method, as reported in a previous paper (8). In addition, this method of injection gave the earliest presentation of polyarthritis. However, the TMJ was not involved with this method. Surprisingly, intradermal injection into the parietal scalp did induce arthritis in both TMJs and cervical vertebrae, although intra-submandibular lymph node injection did not. Arthritis in the TMJ appeared only with our method of intradermal injection into the parietal scalp and was apparent histologically but not clinically. The incidence of arthritis in the TMJs of female Lewis rats immunized via the parietal scalp was

TABLE 1. Failure in induction of arthritis in the TMJ by intra-inguinal lymph node injection

Rat strain	Sex	Immunization[a]		Systemic arthritis			TMJ involvement/ total[d]
		Dose (mg/rat)	Type of adjuvant	Incidence/ total	Mean onset day[b]	Mean high score[c]	
Wistar	F	0.5	Squalene	9/10	10.1	15.3	0/10
Sprague-Dawley	F	0.5	Squalene	10/10	10.2	17.7	0/10
	M	0.5	Squalene	10/10	9.5	18.3	0/10
Lewis	F	0.1	Squalene	10/10	12.3	17.5	0/10
	F	0.5	Squalene	10/10	10.7	19.3	0/10
	F	1.0	Squalene	10/10	9.2	19.4	0/10
	F	0.5	Incomplete Freund adjuvant	10/10	10.3	18.4	0/10
	M	0.5	Squalene	10/10	10.5	19.2	0/10
	F	0	Incomplete Freund adjuvant	0/10			0/10
	M	0	Squalene	0/10			0/10

[a] Each rat was immunized in both inguinal lymph nodes with 0.01 ml of incomplete Freund adjuvant or squalene containing the indicated amounts of human mixed *Mycobacterium* strains (C, DT, and PN).

[b] Mean onset day was calculated by averaging the onset day for each rat per group.

[c] Mean high score was calculated as the arithmetic mean of the highest score of each rat per group, from positive rats only.

[d] Judged by histological findings.

TABLE 2. Dissociated induction of arthritis in various joints with different routes of injection (part 1)

Route of injection	Immunization[a] Dose (mg/rat)	Systemic arthritis			TMJ involvement/ total[d]
		Incidence/ total	Mean onset day[b]	Mean high score[c]	
Footpad	0.5	7/7	13.7	14.1[c]	0/7
Inguinal lymph nodes	0.5	10/10	10.7	19.3	0/10
Tail	0.5	7/7	13.4	14.9[c]	0/7
Posterior cervical region	0.5	8/8	13.8	17.8	0/8
Submandibular lymph nodes	0.5	9/10	11.1	18.9	0/10
Parietal scalp	0.5	10/10	14.1	15.3	3/10
	1.0	10/10	13.9	17.4	6/10
Parietal scalp (squalene alone)	0	0/10			0/10
Control (not injected)	0	0/10			0/10

[a] Each female Lewis rat was immunized with squalene containing the indicated amount of human mixed *Mycobacterium* strains (C, DT, and PN).

[b,c,d] See Table 1, footnotes *b*, *c*, and *d*.

[e] Arithmetic mean of the highest score of each rat per group without scoring the injected foot or tail.

30% with a dose of 0.5 mg per rat and 60% with a dose of 1.0 mg per rat. However, this method produced milder systemic polyarthritis than did other injection routes. Control animals which were sham treated with squalene intradermally into the parietal scalp or not treated at all did not show any arthritis.

Susceptibility of different rat strains to disease via intradermal injection into the parietal scalp. Wistar and Sprague-Dawley rats injected intradermally in the parietal scalp developed mild disease with less than 100% incidence of systemic polyarthritis, whereas Lewis rats developed severe disease with 100% incidence of systemic polyarthritis. Arthritis in the TMJs was induced only in Lewis rats via intradermal injection into the parietal scalp, with 30 to 60% incidence (Table 4).

Effect of unilateral loss of occlusion on disease in TMJs. The animals were divided into seven groups of 10 animals each. Groups 1 through 4 were inoculated with adjuvant, and groups 1, 2, 3, and 6 had a unilateral loss of occlusion. The time of loss of occlusion varied among groups 1 through 3. Groups 1, 2, and 3, which had a occlusal change in addition to adjuvant inoculation, showed a higher incidence of arthritis in both TMJs than group 4, which had adjuvant inoculation alone (Table 5). In particular, groups 1 and 2 showed 100% incidence of arthritis in both TMJs, and group 3 also showed a higher incidence than group 4. However, there was no significant difference in onset and severity of arthritis in the TMJ between the side of the jaw with the teeth removed and the intact side. All of these groups (1 through 4) developed severe systemic polyarthritis with 100% incidence, whereas groups 5 through 7 as control groups did not develop any disease.

Weight curve. The weights of animals in the group with occlusal change only (group 6) did not differ from those of the nontreated group

TABLE 3. Dissociated induction of arthritis in various joints with different routes of injection (part 2)[a]

Joint	Intra-inguinal lymph nodes injection					Intradermal injection into the parietal scalp[b]				
	−	±	+	++	Incidence of arthritis/total	−	±	+	++	Incidence of arthritis/total
TMJ	10	0	0	0	0/10	4	4	2	0	6/10
Cervical vertebra	10	0	0	0	0/10	7	3	0	0	3/10
Shoulder	4	4	2	0	6/10	5	2	3	0	5/10
Elbow	5	4	1	0	5/10	5	3	2	0	5/10
Finger	0	2	3	5	10/10	1	3	5	1	9/10
Hip	0	1	5	4	10/10	3	5	1	1	7/10
Knee	5	2	3	0	5/10	6	3	1	0	4/10
Foot	0	0	0	10	10/10	0	0	2	8	10/10
Coccygeal vertebra	0	1	3	6	10/10	1	2	5	2	9/10

[a] This experiment is the same as that of Table 2. The degree of arthritis was judged by the following histological findings: −, normal joint; ±, mild inflammatory changes; +, severe inflammatory changes; ++, severe inflammatory changes with bone destruction.

[b] Immunization with 0.1 ml of squalene containing 1.0 mg of *Mycobacterium* cells.

TABLE 4. Susceptibility of different rat strains to adjuvant-induced arthritis via intradermal injection into the parietal scalp

Rat strain	Sex	Immunization dose (mg/rat)[a]	TMJ involvement/ total[b]	Systemic arthritis		
				Incidence/ total	Mean onset day[c]	Mean high score[d]
Wistar	F	0.5	0/10	6/10	15.4	9.6
Sprague-Dawley	F	0.5	0/10	9/10	14.7	11.4
	M	0.5	0/10	8/10	15.3	10.3
	M	1.0	0/10	8/10	15.6	9.8
Lewis	F	0.5	3/10	10/10	14.1	15.3
	F	1.0	6/10	10/10	13.9	17.4
	M	0.5	4/10	10/10	13.4	16.2
	M	1.0	5/10	10/10	13.3	16.9

[a] Each rat was immunized intradermally into the parietal scalp with 0.1 ml of squalene containing the indicated amount of human mixed *Mycobacterium* strains (C, DT, and PN).
[b,c,d] See Table 1, footnotes *b*, *c*, and *d*.

(group 7) (Fig. 1). However, there was marked suppression of weight gain in the adjuvant-immunized groups (groups 1 through 4). Furthermore, it was highly interesting that groups with intradermal injection into the parietal scalp showed more suppression of weight gain than groups with intra-lymph node injection.

Histological studies. All rats were sacrificed for histological studies of the TMJ 5 weeks after administration of the adjuvants. To examine local reactions, we sacrificed 20 animals 1 and 3 days and 1, 2, 3, and 5 weeks after adjuvant inoculation into the parietal scalp, and the inoculated skin and regional lymph nodes were examined.

Microscopic changes in the TMJ were somewhat different from those in other joints. The most common change in the TMJ was an inflammatory reaction, which consisted of slight synovitis with small round cells (Fig. 2). The other changes were villous hypertrophy at the tangential zone of the articular cartilage and the disk and, occasionally, adhesion between the articular cartilage and the disk (Fig. 3). The severest changes were thinness and irregularity of the articular cartilage due to disappearance of the radial zone (Fig. 4). The nuclei were enlarged, and the arrangement of cells was not regular in this lesion.

Microscopic changes in the inoculated sites and the regional lymph nodes are shown in Fig. 5 and 6. Both lesions were swollen and reddened upon visual inspection until 2 weeks after inoculation. The microscopic changes included abscess or granuloma formation and continued to the end of the experiment. The changes in the lymph nodes which started after 3 days and continued were enlargement of the paracortical area and irregularity in the arrangement of the germinal center. However, the changes shown Fig. 6 were not always observed in all regional lymph nodes.

TABLE 5. Effect of unilateral loss of occlusion on adjuvant-induced arthritis in the TMJ

Group	Adjuvant inoculation[a]	Loss of occlusion[b]	TMJ involvement/ total[c]	Systemic arthritis	
				Incidence/ total	Mean high score[d]
1	Yes	Yes (2 weeks before adjuvant inoculation)	10/10	10/10	17.7
2	Yes	Yes (simultaneously)	10/10	10/10	17.3
3	Yes	Yes (2 weeks after adjuvant inoculation)	7/10	10/10	16.8
4	Yes	No	6/10	10/10	17.4
5	Oil alone	No	0/10	0/10	
6	No	Yes	0/10	0/10	
7	No	No	0/10	0/10	

[a] Each rat (group 1 through 4) was immunized intradermally in the parietal scalp with 0.1 ml of squalene containing 1.0 mg of human mixed *Mycobacterium* strains (C, DT, and PN).
[b] See text.
[c,d] See Table 1, footnotes *c* and *d*.

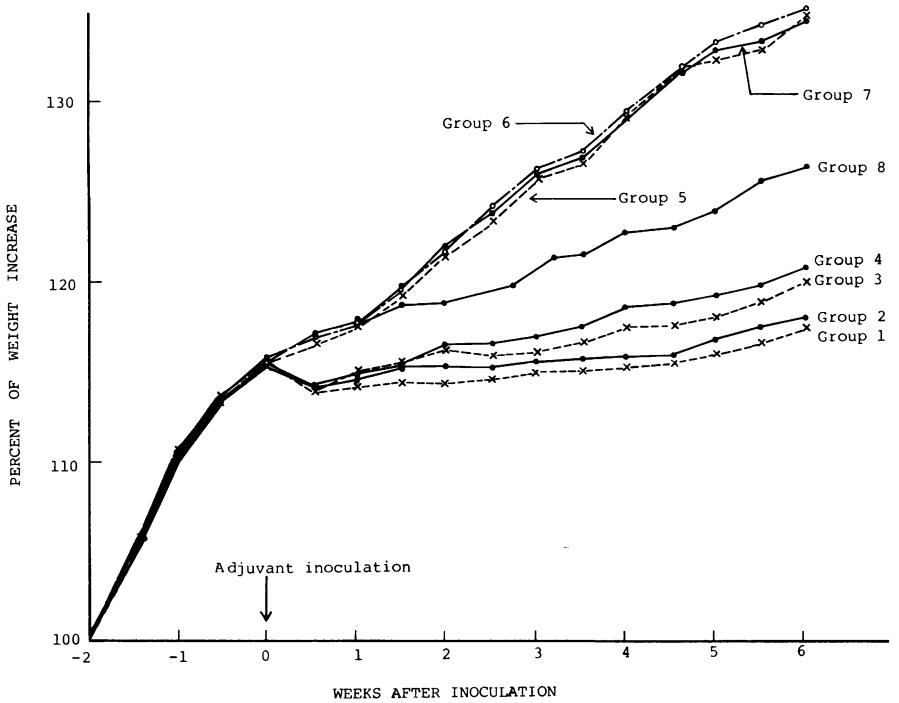

FIG. 1. Weight gain curve for rats suffering from adjuvant-induced arthritis in the TMJ. Each experimental group corresponds to that in Table 5 except group 8, members of which were immunized in both inguinal lymph nodes with the same quantity of adjuvant.

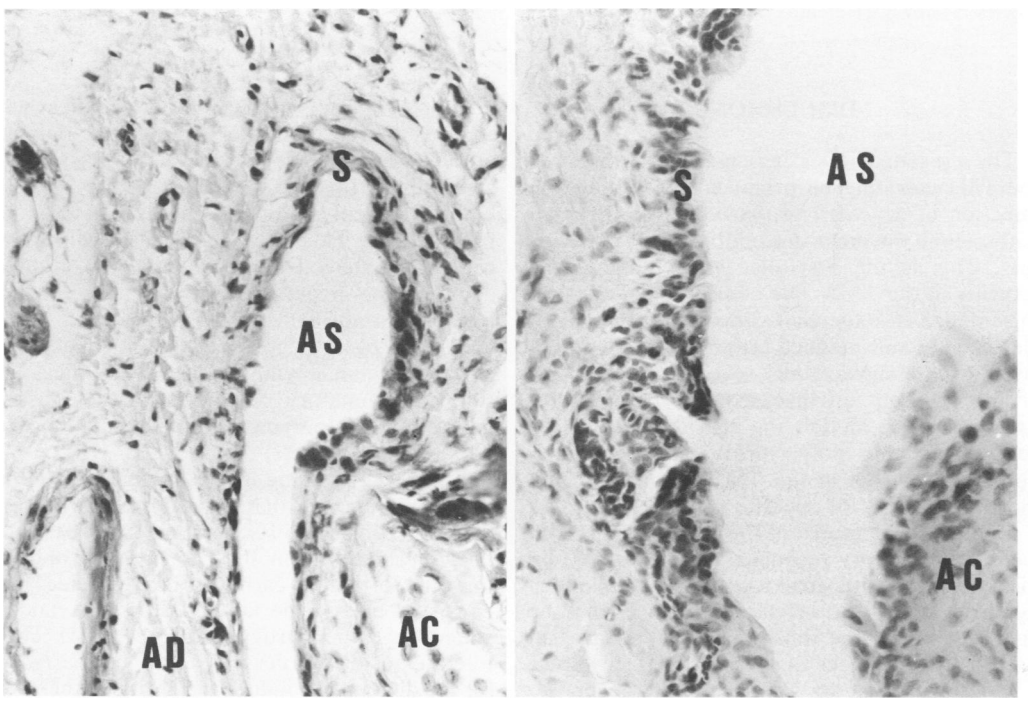

FIG. 2. Section of arthropathy in the TMJ 5 weeks after adjuvant inoculation intradermally into the parietal scalp (at right), compared to normal TMJ (at left). Infiltration of small round cells at the synovialis and enlargement of articular space are shown. (Hematoxylin and eosin; ×270) S, Synovialis; AS, articular space; AD, articular disk; AC, articular cartilage.

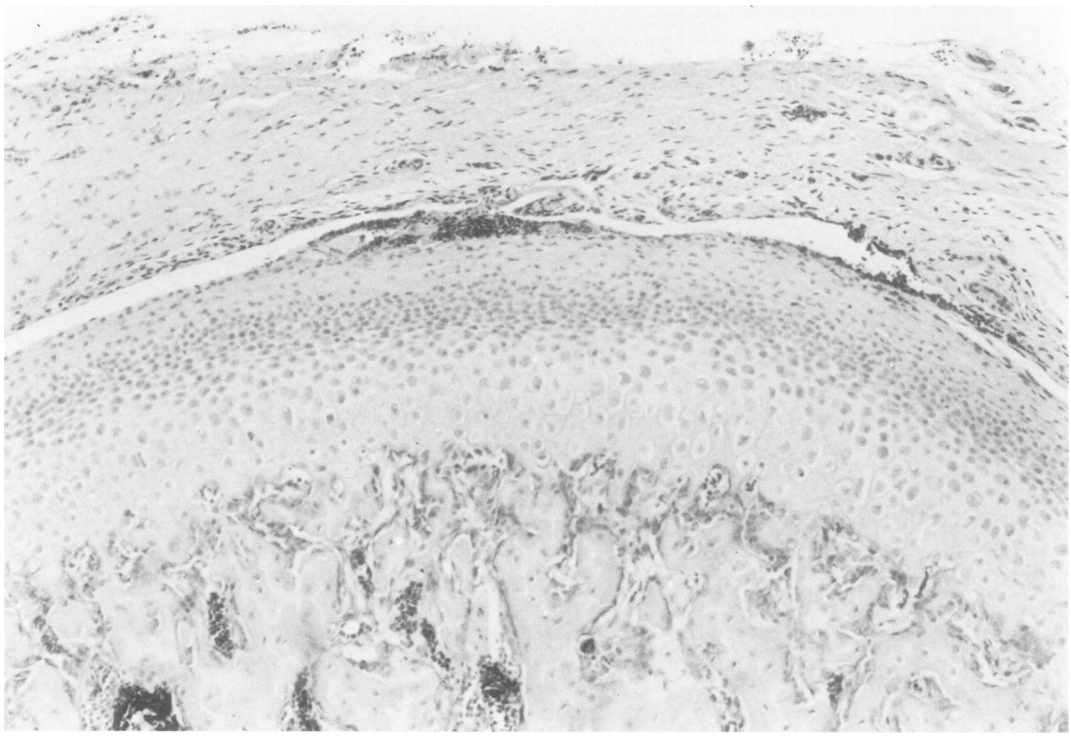

FIG. 3. A TMJ 5 weeks after adjuvant inoculation intradermally into the parietal scalp. There is villous hypertrophy at the tangential zone of the articular cartilage and the disk. In this section, adhesion between both tissues is shown. (Hematoxylin and eosin; ×140)

DISCUSSION

The present studies indicate that arthritis in the TMJ can only be produced by intradermal injection of *Mycobacterium* cells into the parietal scalp in the most susceptible strain of Lewis rats. This is the first successful induction of arthritis in the TMJ. The clinical appearance of this model is somewhat different from that of classic adjuvant-induced arthritis. Rats with the latter disease showed very severe systemic polyarthritis without any disease in the TMJ, whereas in this new model, the rats developed less severe systemic polyarthritis with 60% incidence of arthritis in the TMJ, including much more severe loss of appetite and weight loss.

Histological features of this model in the TMJ were inflammatory responses, characterized by mild synovitis with small round cells, and villous hypertrophy of the articular cartilage and the disk. However, the inflammatory responses in the TMJ were milder than those in other joints.

As far as we know, there have been no previous reports about the method of intradermal injection into the parietal scalp in experimental models of arthritis. The present studies revealed the superiority of this method for induction of arthritis in the TMJ. It is unclear why arthritis in the TMJ can be induced only by intradermal injection into the parietal scalp. We suppose that this may be due to greater accumulation of mycobacterial fragments in tissues adjacent to the TMJ (13), because this disease is generally believed to be one manifestation of delayed-type hypersensitivity to disseminated mycobacterial fragments (17). In support of this idea, histological features in the inoculated skin and the regional lymph nodes were local and acute inflammatory responses with a massive infiltration of polymorphonuclear leukocytes around lipid droplets.

Taguchi (15), using the scanning electron microscope, reported that the articular cartilage in the TMJ consisted of several layered structures of collagen fibrils in the transitional zone but that the articular cartilage in the knee joint consisted of a single layered structure, and he stated that this structure of the TMJ could tolerate various forces. This may be the reason for the difficulty of inducing arthritis in the TMJ.

It was of interest that occlusal change in addition to adjuvant inoculation markedly increased the incidence of arthritis in the TMJ. This enhancement might be due to weak but

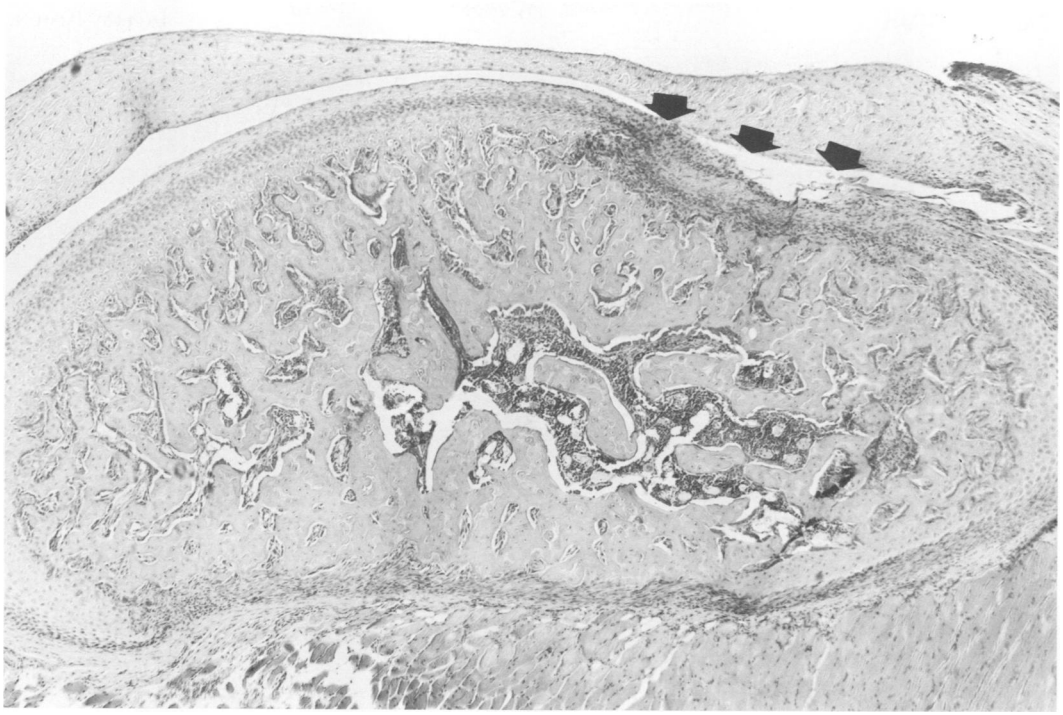

FIG. 4. A TMJ 5 weeks after adjuvant inoculation intradermally into the parietal scalp. The most prominent feature is thinness and irregularity of the articular cartilage due to disappearance of the radial zone (arrows). (Hematoxylin and eosin; ×55)

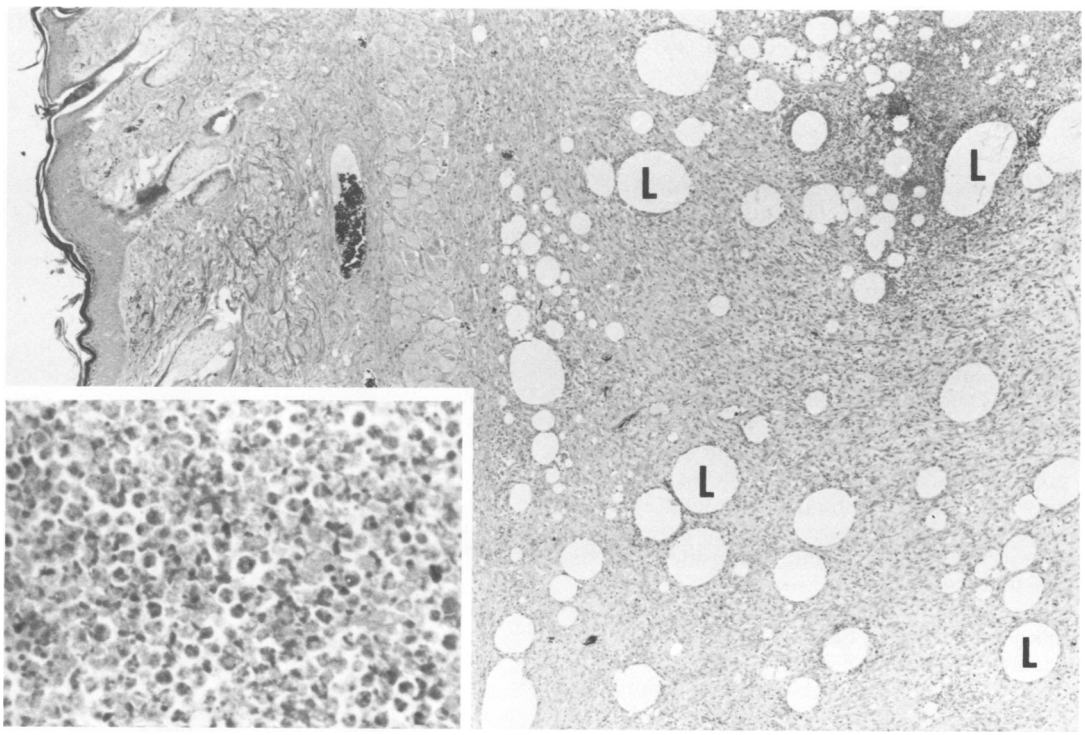

FIG. 5. Inoculated skin 2 weeks after adjuvant inoculation intradermally into the parietal scalp. There is granulation tissue around numerous lipid droplets (L) at the subcutaneous tissue level (×55). An enlarged feature of the granulation tissue is shown in the inset (×400). Heavy infiltration of polymorphonuclear leukocytes can be seen. (Hematoxylin and eosin)

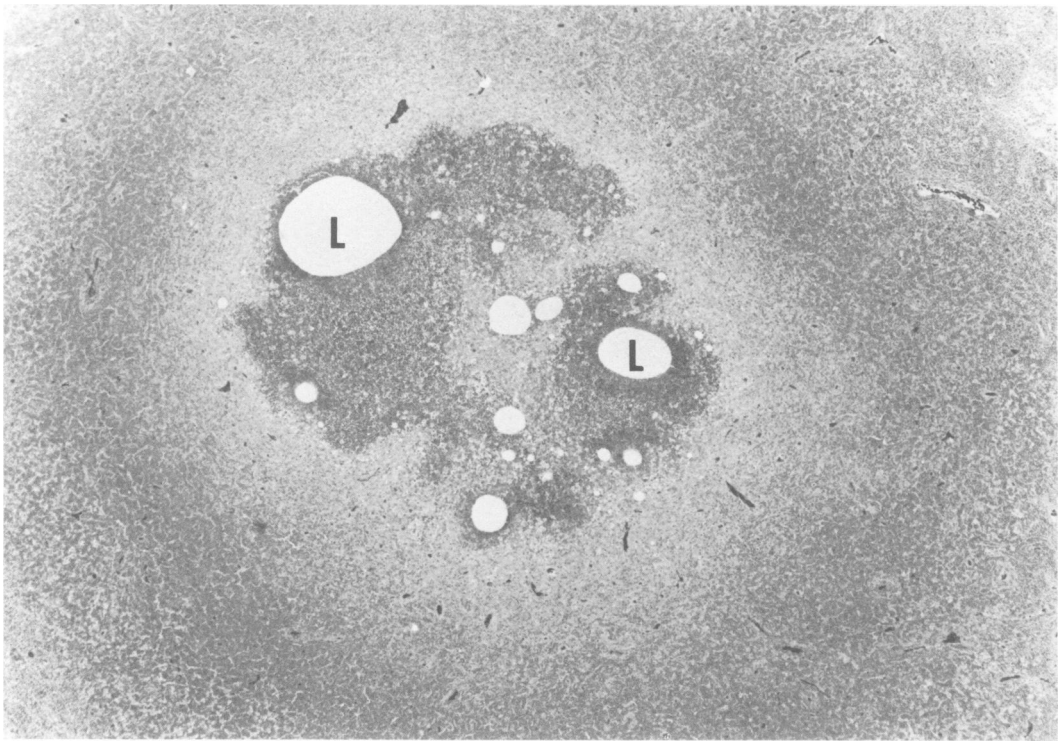

FIG. 6. A regional lymph node 2 weeks after adjuvant inoculation intradermally into the parietal scalp. Granulation tissue can be seen around lipid droplets (L), with numerous capillaries and heavy infiltration of lymphocytes. (Hematoxylin and eosin; ×55)

continuous loads on the TMJ, as reported by Glenn and Gray (7). They studied the effect of local trauma on adjuvant-induced arthritis and found that manually twisting the tibiotarsal joint alone did not induce arthritis, but did potentiate the adjuvant-induced lesion. So far as the TMJ was concerned, Furstman (6) reported that the loss of occlusion induced a thinning of the articular cartilage as well as severe osteosclerotic changes in both TMJs. These histological changes were not observed to any degree in the present studies, but no significant difference between the side of the jaw with teeth removed and the intact side were observed as well as in his report. The jaw might be considered as one functional unit.

Finally, the TMJ is a complex joint, and only this joint mediates the unique forces of dental occlusion. There are fewer studies concerning the TMJ than there are for other joints, and additional studies are required to elucidate the pathogenesis of this model. This model can be applied to various experiments, and it is expected that studies concerning this joint will progress.

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