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THE PATHOGENESIS OF ADJUVANT DISEASE IN THE RAT

I. A Histologic Study of Early Lesions in the Joints and Skin†

Some years ago Pearson described a polyarthritis, accompanied by iridocyclitis and mucocutaneous lesions, occurring in rats injected with standard mycobacterial adjuvant mixtures.[1,2] This experimental process has interest as a possible model for certain human diseases of unknown origin, notably Reiter's syndrome and an ill-defined group of parainfectious arthritides.[3] Its clinical features have been described in some detail; and with Pearson we have published a histologic description of the fully developed lesions in the joints, eye and other tissues.[3,4,5]

At the same time, evidence was advanced which favors the view that adjuvant disease may depend upon a hypersensitive reaction of the cellular (delayed) type to antigens of the injected tubercle bacilli, of the host's connective tissue, or some complex of the two.[6] Indeed we have been able to transfer the experimental disease to normal rats with living lymph node cells from sensitized donor rats of the same inbred strain.[7] A close similarity of adjuvant disease to experimental autoallergic encephalomyelitis, in which lesion formation appears to be based on a hypersensitivity of the cellular type directed at antigenic constituent(s) of normal myelin, has been apparent with regard to each experimental and morphologic property investigated thus far.[6,7]

The only attempt to elucidate the nature of the earliest lesions of adjuvant disease is reported in a recent study by Jones and Ward.[8] These authors observed focal accumulations of polymorphonuclear leukocytes in the lung, spleen, and other viscera, as well as in the joint synovia and periarticular tissues of rats injected with adjuvant. These changes appeared at 5 to 7 days. The visceral foci were rapidly replaced by granulomatous lesions,

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particularly in the lung, while the joint lesions continued for some time to be primarily purulent. Later changes included a proliferative connective tissue response, the formation of lymphocyte aggregates, bone destruction, and periosteal new bone formation. Joint disease did not become grossly apparent till 10 to 14 days in the majority of the animals.

From these observations, Jones and Ward[8] inferred that an infectious agent might be responsible for initiating the lesion formation of adjuvant disease. However, despite numerous attempts, it has not proven possible to isolate pathogenic bacteria, *Mycoplasmataceae,* or viruses consistently from the blood or affected joints of rats with early or full-blown disease; nor could disease be transferred with blood or diseased tissue to normal animals.[2,9] Large doses of a variety of antibiotics have proven ineffectual in suppressing actively induced arthritis.[2] Perhaps the strongest argument against infection as the basis of lesion formation in this process is provided by the passive transfer experiment, which succeeds only when rats of the same inbred strain are used as donor and recipient and only when living cells of lymphoid tissue are transferred. Furthermore, there is an apparent inconsistency between the character of the early lesion, as described by Jones and Ward,[8] and the early lesions of autoallergic encephalomyelitis or other reactions of cellular hypersensitivity, which begin quite uniformly with perivascular infiltration of hematogenous mononuclear cells.[10,11]

It seemed of some importance, for these reasons, to extend the study of the early lesion of adjuvant disease. In the present paper, we report the results of a conventional histologic examination of joint lesions and the easily accessible ear nodules in animals sacrificed at varying times in relation to the clinical onset. A second paper[12] describes the results of an attempt to identify the source of the infiltrating cells in both joint and ear lesions by the use of tritiated thymidine and radioautography.

METHODS

Full details of the method of producing and evaluating disease are given in earlier publications.[4,6,9] For the purposes of the present study, female Sprague-Dawley rats, obtained from the Charles River Breeding Laboratories, Brookline, Massachusetts, and weighing 150-200 gm., received a single foot-pad injection (0.05-0.10 ml.) of a suspension of heat-killed tubercle bacilli in mineral oil, 3 mg/ml. All rats were examined daily following injection for the appearance and severity of arthritis, ear nodules, or eye changes. Animals were sacrificed at arbitrary intervals before the expected onset of clinically apparent disease, at onset, or at fixed times thereafter. All extremities, whether or not they showed gross evidence of disease, and both ears were examined histologically after fixation in formalin, decalcification in EDTA, paraffin embedding, and conventional sectioning and staining with hematoxylin and eosin. The inoculated foot and viscera were not examined.

Lesions were evaluated in terms of their size, the proportion of different types of mononuclear cells in the inflammatory infiltrates, the presence of polymorphonuclear leukocytes and necrosis, and the proliferation of fixed tissue elements such as fibroblasts, epidermal cells (in the skin adjacent to ear nodules), and synoviocytes (in the presence of joint inflammation). The percentage figures given in the tables are based, in the case of lymphocytes, monocytes, and histiocytes, on the total number of mononuclear cells counted. The figures for polymorphonuclear leukocytes are based on total infiltrating cells. The presence of necrosis and fibroblastic, synoviocytic, or epidermal proliferation is indicated by +, and their absence is noted by no indication, all slides having been carefully examined for each of these changes. For certain animals, separate counts of several lesions are given.

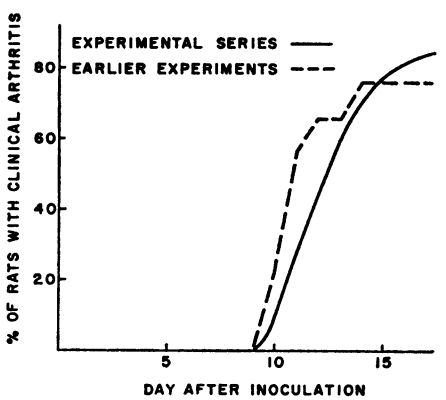

FIG. 1. Cumulative incidence of gross arthritic manifestations in animals of the present experimental series, compared with those of experiments described in earlier reports.

RESULTS

Two series, including 30 and 41 animals respectively, were used in the present experiments. These were also employed in the radioautographic study reported in the next paper.[18] As shown in Figure 1, clinical disease first appeared on the tenth day following injection of adjuvant, in about one fifth of the animals in both series. By 14 days, over three quarters of the animals showed grossly apparent lesions. These incidence figures agree well with those obtained in earlier experiments (Fig. 1). The study of tissues from rats included in the two experimental series but sacrificed before the onset of clinical disease was based on the assumption that at least three quarters of such animals would subsequently develop disease. Histologic lesions of the skin or joints were in fact present in almost all animals autopsied at ten days or later. On day nine, 5 out of 10 rats examined showed definite lesions. No histologic abnormality could be

recognized in animals autopsied before nine days. Ear nodules tended to appear somewhat later than lesions of the extremities. Of 18 rats sacrificed at 12 days, 14 showed clinical joint disease, but only 6 had developed grossly apparent ear nodules. However, lesions were found histologically in ear sections from 14 of the 18 animals.

Adjuvant disease has been reported to consist of irregularly disseminated, focal, infiltrative lesions in certain connective tissues, notably those associated with joints and tendons, the glabrous skin, and the ciliary body of the eye,[8] and these findings were confirmed in the present study. For convenience, we will present numerical data obtained by studying lesions in the joint and tendon synovia, in tendons and ligaments, in the loose periarticular, periosteal, and subcutaneous connective tissues of the extremity, and in the subcutaneous tissue of the ear (ear nodules) separately in the following text. This distinction is arbitrary, however, as these regions are not sharply separated, the synovia merging imperceptibly with the periosteal and periarticular connective tissue, the tendons and ligaments with the periosteum, etc. It was in fact observed that lesions may occur in any one of these regions without being found in the others or may appear as multiple simultaneous foci in histologically distinct regions of the extremity. The basic character of the lesions observed appeared to be independent of their location and to depend rather on their age and size. With the passage of time, an increase was found not only in the number of extremities involved and in the number of joints involved in an extremity but also in the number of anatomic regions affected in relation to any one joint and in the number and size of the lesions in any particular region such as the synovia. It is clear, therefore, that in disease of several days duration both old and new lesions may be present in a single area.

The principal elements of the infiltrative lesions were mononuclear cells, which presented the same characteristics as the infiltrating cells observed in lesions of autoallergic encephalomyelitis[11,13] and in skin reactions of delayed hypersensitivity.[10,14] They were classified as *medium-large lymphocytes,* cells 7-11μ in diameter with indented or lobulated nuclei, having a dense nuclear membrane and one or more prominent nucleoli, and a variable degree of cytoplasmic basophilia; *monocytes,* cells generally somewhat larger than the lymphocytes with paler nuclei, usually horseshoe-shaped or pleomorphic, no nucleoli, and little or no cytoplasmic basophilia; and *histiocytes,* cells of the same type, usually more than 12μ in diameter, with oval or pleomorphic nuclei. These appeared to make up a continuous spectrum from small cells, difficult to distinguish from small lymphocytes, to large histiocytic cells of almost epithelioid character.

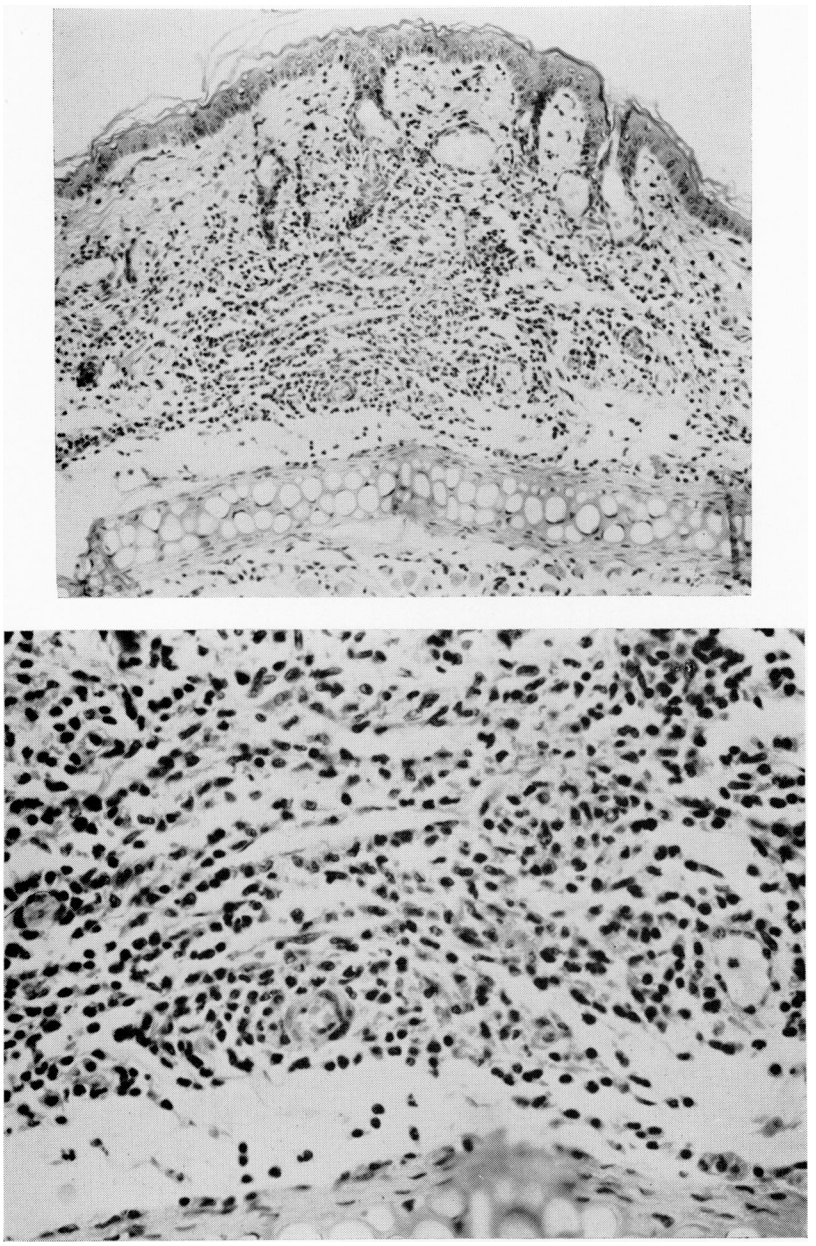

FIG. 2. Early ear nodule in rat sacrificed at ten days at onset of arthritis. Infiltrate is perivenous and is made up entirely of mononuclear cells resembling lymphocytes. Hematoxylin-eosin, X160 and X400.

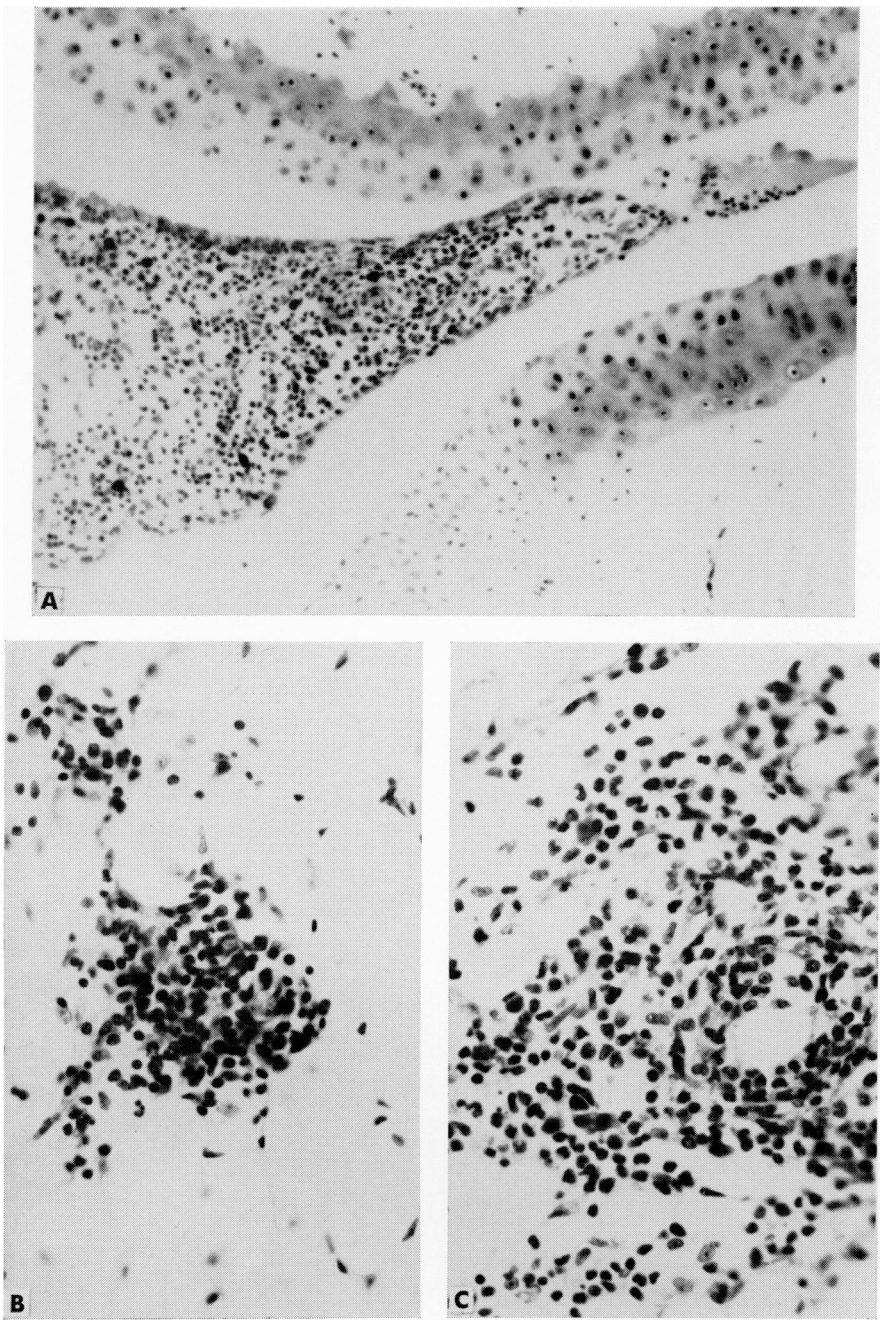

FIG. 3. Early synovial lesions.

A. Metacarpophalangeal joint in left forefoot of rat sacrificed at 11 days, at onset of grossly visible arthritis.

B. Right wrist of rat sacrificed at ten days, before onset.

C. Right ankle of another rat sacrificed at ten days, before onset.

All show perivenous mononuclear cell infiltration. A few polymorphonuclears are present in lesion shown in C.

A. Giemsa, X160. B and C. Hematoxylin-eosin, X400.

Radioautographic evidence, presented in the next paper,[19] suggests that all or almost all the large cells are in fact derived from smaller lymphocytic precursors. The boundaries between the three classes are therefore quite arbitrary. However, counts based on the above criteria could be reproduced by two different observers, differing by no more than 10 to 20 per cent. They have proved useful as a means of characterizing the cell population in lesions at different moments in their evolution. A shift with age from predominantly lymphocytic lesions to lesions in which monocytic or even histiocytic elements predominated was apparent in all the different anatomic areas (*vide infra*).

The infiltrating mononuclears, especially in early lesions, frequently showed a striking perivascular localization. This finding suggests that these cells are hematogenous, a suggestion supported by the radioautographic data.[19] The perivascular relationship tended to be obscured in older lesions although, even in diffuse lesions, perivascular lymphocytic cuffing sometimes remained quite apparent.

Necrosis was observed only in the center of relatively large lesions, usually of one or more days probable duration. The necrotic zone was characteristically surrounded by a zone in which histiocytes were the main infiltrating cells and in which fibroblastic proliferation was evident. This in turn was surrounded by a zone of lymphocytic infiltration, in part perivascular.

Foci of necrosis were in general filled with polymorphonuclear leukocytes. However, in many older lesions, polymorphonuclears were present as a diffuse infiltrate, apparently superimposed on the characteristic mononuclear lesion, in the absence of any evidence of necrosis. In the synovial fluid these cells predominated only when they were numerous in the synovial lesions. They were largely or entirely absent from the earliest lesions examined, with two exceptions. One of ten animals examined at nine days showed a single necrotic focus filled with polymorphonuclears in the subcutaneous connective tissue of one forelimb. Another rat with intense lesions at nine days showed diffuse polymorphonuclear infiltration amounting in some areas to 10 to 20 per cent of the infiltrating cells. Deposition of "fibrinoid" was seen in vascular walls and extravascular tissue spaces in many lesions, especially those containing polymorphonuclear cells. No attempt was made to quantitate this change.

Proliferation of fibroblasts was apparent in zones of infiltration, especially in lesions of some days duration. Similarly, synovial lining cells (synoviocytes) underwent active proliferation in older joint or tendon lesions. The skin above ear nodules showed epidermal thickening, which also

TABLE 1. CYTOLOGIC ANALYSIS OF EARLY DEVELOPMENT OF EAR NODULES

Day of onset/sacrifice	Average diameter of lesion (X 100 μ)	Predominantly perivascular localization	Percentage of infiltrating mononuclear cells			Presence in lesion of			
			Lympho-cytes	Mono-cytes	Histio-cytes	Polymorpho-nuclear infiltration	Necrosis	Fibroblas-tic proli-feration	Epidermal thickening
Before onset of clinical disease									
-/12	1	+	70	22	8				
-/12	1.5	+	95	5	0				
-/12	3	+	93	7	0				
Before onset of clinical disease in ear, after onset in extremities									
12/12	1	+	90	10					
12/12	1	+	80	18	2				
12/12	1.5	+	85	10	5	5%	+		+
11/12	3		71	21	8				
12/12	4		86	9	5	2%			
10/14	7.5		necrotic zone			55%	+	+	+
			25	30	45			+	+
			70	15	15			+	+
At onset of disease in ear									
10/10	2		80	10	10				
10/10	2		60	25	15			+	
10/11	3		81	14	5				+
	3		71	12	17				
	4		82	12	6				
11/11	4		41	25	34			+	
12/12	4	+	90	8	2				
	Diffuse		A few lymphocytes peripherally			90%	+	±	
12/12	8	+	80	15	5	20%	+	+	+
12/12	10	+	85	15	0				+

One day after onset in ear									
10/11	1.5		61	20	19				
	3.5		45	26	29				
	5		50	26	24	5%			+
10/11	2.5		33	36	31	5%	+	+	+
	8		57	25	18				+
11/12	2.5		42	47	11				
	3		44	28	28	45%	+		+
	3.5		80	12	8				
	6		83	16	1				
	14		necrotic zone			45%	+		+
			23	20	57			+	+
			85	10	4				
Two days after onset in ear									
11/14	1	+	100	0	0				
	2.5		44	21	35				
	4		69	29	2	5%	+		
	4		66	19	15				
	5		83	11	6	3%	+		
Three days after onset in ear									
10/13	4		79	15	6				
	6		80	14	6				
	10		70	24	6				+
11/14	5	+	100	0	0				
	10		25	30	45	2%	+	+	+
12/15	3		72	24	4				
	3		70	20	10				
	3		54	26	20				
	4		74	20	6				
	8		40	23	37			+	+
14/17	8		69	15	16	2%	+	+	+
14/17	10		68	18	13				
14/17	12		60	30	10	5%	+		

appeared related to the age and size of the lesions. These changes were clearly secondary to the infiltrative lesions, and are noted separately in the tables.

Destruction of bone or cartilage and formation of pannus were not observed in early lesions. Since these changes are commonly seen in joints which have shown grossly visible abnormality for at least 1 to 2 days,[8] they were felt to be secondary to one or more of the other changes observed. The mechanism whereby they are brought about is itself an important question meriting separate investigation.

Ear nodules (Fig. 2). Data concerning ear nodules are presented first, since these lesions tended to remain circumscribed and appeared less complex anatomically than those affecting the extremities. Also, minimal lesions of the ear were easier to observe clinically; it was felt, therefore, that the timing of lesion formation in relation to overt clinical disease in the ear was more precise than in the joints. Nodules normally appear in the ears a day or two later than lesions in the extremities.

As shown in Table 1, the earliest lesions, in animals sacrificed before the appearance of grossly recognizable ear nodules or just at onset, appeared to be perivascular cuffs of mononuclear cells, principally lymphocytes. These were associated with congestion of small veins and arteries. By 1 to 2 days after clinical onset more than half the cells, in many cases, were monocytic or histiocytic. Later, there appeared to be a relative increase again in forms resembling lymphocytes. Polymorphonuclears, with or without frank necrosis, were not found in the earliest lesions. In lesions studied after the clinical onset they varied from 0 to 90 per cent of the infiltrating cells and showed a correlation with the size and intensity of the lesions. Similarly, fibroblastic proliferation and epidermal thickening over the nodules were absent from the earliest lesions, were most conspicuous in relation to large lesions, and were correlated with the presence of polymorphonuclears and necrosis.

Lesions in joint and tendon synovia (Fig 3). As shown in Table 2, lesions in the synovia also begin most commonly as circumscribed perivascular infiltrates; but by one or more days after the onset of clinical abnormality, at which time many lesions are presumably 2 to 3 days old, most have become diffuse, though perivascular cuffing remains evident. The same sequence was observed here as in the ear nodules, lymphocytes predominating in the earliest lesions and monocytes and histiocytes by 1 to 3 days after clinical onset. Again polymorphonuclear infiltration and foci of necrosis were absent in the earliest specimens but appeared in

increasing numbers within a day or two; the presence of polymorphonuclears in the synovial fluid was correlated with their presence in large numbers in the lesion itself. Lesions containing distinct concentric zones of necrosis, of histiocytes, and of lymphocytes were seen in animals with disease of several days duration. Synovial cell proliferation, like epidermal proliferation adjacent to ear nodules, appeared to reflect the age and, to a lesser extent, the intensity of the infiltrative lesions.

Tendon and ligament lesions. The same elements were observed in lesions appearing within the dense connective tissue of tendons and ligaments (Table 3). Here, however, polymorphonuclear infiltration tended to be more extensive, while focal lesions with separate zones of necrosis and histiocytic and lymphocytic infiltration were uncommon. Fibroblastic proliferation was observed frequently in lesions with a probable age of more than 1 to 2 days.

Lesions in periarticular, periosteal, and subcutaneous connective tissue. As in the other anatomic regions examined, the earliest lesions were perivascular aggregates of lymphocytes (Table 4). However, in several rats, a diffuse infiltration of lymphocytes without obvious perivascular localization was the first change noted. Again, a shift in the nature of the cell population with time was observed, increasing numbers of cells being characterized as monocytes or histiocytes in older lesions. Here too, polymorphonuclears were completely absent from the earliest lesions. Zonal lesions were common after the first day, often with histiocytes and fibroblastic proliferation in the center and lymphocytes at the periphery.

DISCUSSION

Adjuvant disease in the rat consists of disseminated focal lesions in connective tissue, principally of the extremities, the uveal tract, and the glabrous skin. The present report is concerned with the pathogenesis of lesions in the extremities (arthritis, periostitis, tenosynovitis) and skin (ear nodules). The character of lesion formation in the eye will be the subject of a separate report.[15] The findings at all sites examined have been closely similar and permit a general statement as to the nature of the basic lesion in this process.

The earliest lesion of adjuvant disease in our material appears to be a perivascular accumulation of mononuclear cells with the morphologic character of medium-sized lymphocytes, accompanied by venous congestion. The process evolves by an increase in the number of lesions, both at previously involved sites and at new sites, an increased number of cells in

TABLE 2. CYTOLOGIC ANALYSIS OF EARLY LESIONS IN JOINT AND TENDON SYNOVIA

Day of onset/sacrifice	Average diameter of lesion* (X 100 μ)	Predominantly perivascular localization	Percentage of infiltrating mononuclear cells			Presence in lesions of			Predominant cell type in synovial fluid
			Lympho-cytes	Mono-cytes	Histio-cytes	Poly morpho-nuclear infiltration	Necro-sis	Synovial cell pro-lifera-tion	
Before onset of clinical disease									
-/9	1	+	100	0	0				
-/9	D		100	0	0				
-/9	1	+	85	15	0	3%		+	
-/9	2	+	95	5	0			+	
-/10	1	+	100	0	0				
-/10	D		95	5	0				
-/10	1	+	90	9	1	2%			
-/10	1.5	+	80	15	5	1%			
-/11	1	+	93	7	0	5%		+	
-/11	1.5	+	95	5	0				
-/11	1	+	95	4	1				
-/11	1.5	+	70	20	10				
At onset of disease									
10/10	1	+	93	7	0			+	
10/10	1.5	+	100	0	0				
10/10	D		80	15	5	30%	+	+	
11/11	1	+	94	6	0				
11/11	D		54	22	24	30%		+	polymorphonuclear

One day after onset									
10/11	D		+	95	5	0		+	
10/11	D		+	94	4	2	10%	+ +	polymorphonuclear
10/11	2		+	85	14	1		+	
	D		+	40	20	40		+	
10/11	1.5			36	20	44	20%	+	
	D		+	13	20	67	2%	+ +	polymorphonuclear
11/12	D			18	32	50		+	
Two days after onset									
11/14	D		+	85	11	4	5%	+	lymphocyte
Three days after onset									
11/14	D		+	75	21	4		+	
	D	central		50	30	20	40%	+ +	polymorphonuclear
		peripheral	+	85	10	5		+	
12/15	2			29	38	33		+	
	D	central		32	18	50	90%	+ +	
		peripheral	+	82	12	6	6%	+	
14/17	D			81	19	0			

* Diffuse or poorly circumscribed lesions designated "D."

TABLE 3. CYTOLOGIC ANALYSIS OF EARLY TENDON AND LIGAMENT LESIONS

Day of onset/sacrifice	*Average diameter of lesion* (X 100 μ)*	*Predominantly perivascular localization*	*Percentage of infiltrating mononuclear cells*			*Presence in lesions of*		
			Lympho-cytes	*Mono-cytes*	*Histio-cytes*	*Polymorpho-nuclear infiltration*	*Necrosis*	*Fibroblastic proliferation*
Before onset of clinical disease								
-/9	0.5	+	100	0	0			
-/9	D	+	96	4	0			
-/10	1	+	100	0	0			
-/11	1	+	85	14	1	15%		
-/11	2	+	96	3	1			
At onset of disease								
10/10	D		94	4	2	30%	+	+
10/10	D		90	7	3			+
11/11	D		87	11	2	30%	+	
	D		58	24	18	80%	+	+
	D		58	24	18			+
	D		50	34	16	25%	+	
One day after onset								
10/11	D	+	96	4	0			
	D		95	4	1			
	D		80	10	10			
10/11	D		68	26	6	4%		
	D		62	30	8			

10/11	2			81	12	7	20%		
	D			80	16	4			
	D		+	56	21	23	10%	+	+
	D		+	10	32	58	3%	+	+
11/12	D			85	14	1	20%		
10/13	D		+	85	9	6	1%	+	+
10/13	D			86	8	6			
	D			54	36	10	30%		
Two days after onset									
10/13	D			54	28	18			+
11/16	D			64	32	4			
	D	central		37	36	27			
		peripheral	+	95	4	0			
Three days after onset									
10/13	D		+ peripheral	75	19	6			
10/13	D			54	30	16	25%	+	+
11/14	D		+	95	4	1			
	D			44	38	18			
11/14	D		+ peripheral	85	10	5			
	D			57	29	14	40%		
12/15	D			60	29	10			

* Poorly circumscribed lesions designated "D."

TABLE 4. CYTOLOGIC ANALYSIS OF EARLY LESIONS IN PERIARTICULAR, PERIOSTEAL, OR SUBCUTANEOUS CONNECTIVE TISSUE

Day of onset/sacrifice	*Average diameter of lesion* (X 100 μ)*	*Predominantly perivascular localization*	*Percentage of infiltrating mononuclear cells*			*Presence in lesions of*		
			Lympho-cytes	*Mono-cytes*	*Histio-cytes*	*Polymorpho-nuclear infiltration*	*Necrosis*	*Fibroblastic proliferation*
Before onset of clinical disease								
-/9	1	+	100	0	0			
-/9	D		100	0	0			
-/9	2	+	94	6	0			
-/10	1	+	95	4	1			
-/10	D	+	95	5	0			
-/10	D	+	90	6	4	3%		
-/10	3	+	76	20	4			
-/11	2	+	96	4	0			
-/11	4	+	70	12	18			
At onset of disease								
10/10	2	+	100	0	0			
10/10	D		85	12	3			
10/10	D	+	91	9	0			+
10/10	D		84	10	6			
	D		53	27	20	25%	+	
11/11	D central		10	30	60			+
	peripheral	+	70	20	10			
One day after onset								
10/11	4	+	91	6	3	1%		
	D		80	10	10			

10/11	5		+	90	6	4			
	D		+	91	7	2			
	D		+	63	36	1	2%	+	+
10/11	D	central		56	32	12			+
		peripheral		90	8	2			
10/11	10			75	10	15			
	D			81	15	4	25%		
11/12	D			54	24	22			
11/12	4			70	24	6			
	D			32	18	50			
10/14	D			70	25	5			
Two days after onset									
10/13	D	central		54	28	18			+
		peripheral	+	98	0	2			
Three days after onset									
10/13	D	central		26	28	46	40%	+	
		peripheral	+	75	19	6	25%	+	
11/14	D			44	38	18			
14/17	D		+	33	19	48	25%		

* Poorly circumscribed lesions designated "D."

the lesions, which thereby become larger or more diffuse, and a change in the character of the cell population, monocytic or histiocytic elements tending to become predominant, especially in the center of large focal lesions. Radioautographic evidence, summarized in the next paper,[12] shows that the lymphocytes are largely or entirely hematogenous and that an appreciable proportion of the monocytes and histiocytes are directly derived from these hematogenous cells.

Several further changes are common in the lesions of adjuvant disease; specifically, diffuse and focal infiltrations of polymorphonuclear leukocytes, focal necrosis, and proliferation of fibroblasts, synoviocytes, and epidermis adjacent to infiltrative lesions. In many cases, these are present at the time of clinical onset of disease. However, they are judged to represent secondary alterations since they were uniformly absent from the earliest lesions examined, were never found in the absence of the characteristic mononuclear cell infiltrates, and were frequently lacking even in florid older lesions. Such changes as destruction of bone and cartilage and formation of pannus are first noted after the clinical onset and must also be regarded as secondary to one or more of the other changes described.

It will be noted that the appearance of clinical disease is a misleading index to the initiation of lesion formation, since lesions are found histologically one or more days before clinical onset. Indeed, by the time of clinical onset the lesions observed include some which are new and others which are old in the immunologic sense. A tuberculin reaction reaches its peak by 24 to 48 hours. The adjuvant disease lesion must be considered in somewhat the same light; only changes observed in the first few hours may properly be considered "early."

Our findings conflict with those of Jones and Ward.[8] These authors may have been unsuccessful in examining a large number of joint sections taken at the crucial moment, a day or two before the appearance of clinical disease. They were perhaps unduly impressed by visceral foci of polymorphonuclear cells, appearing during the latent period after adjuvant inoculation and attributable to direct embolization by droplets of the injected adjuvant. Since they worked with a different strain of rat and used a different adjuvant mixture and route of inoculation than were employed in our study, they may even have been dealing with a somewhat different process. In particular, their use of olive oil in the adjuvant may have resulted in an overwhelming polymorphonuclear response to minimal emboli of injected material. Finally, it is possible that, in the presence of large numbers of polymorphonuclears, they overlooked an underlying mononuclear cell response. While we did not examine the viscera, it is noteworthy

that foci of necrosis or aggregates of polymorphonuclears in the region of the joints and long bones or in the ear, were entirely lacking in animals autopsied at nine days or earlier with one exception. Nevertheless, at nine days, half the animals examined already showed mononuclear infiltrates, almost purely lymphocytic in character.

Our microscopic findings are consistent with the thesis that the adjuvant disease lesion represents a reaction of cellular hypersensitivity to an as yet undefined antigen. Its histologic characteristics are closely similar to those of known lesions of cellular hypersensitivity such as the delayed skin reaction and first-set skin homograft rejection.[10, 14] In particular the close analogy of adjuvant disease and experimental autoallergic encephalomyelitis is extended. That process also begins[11, 18] with perivascular aggregations of hematogenous lymphocytes and continues with a progressive increase in the number of lesions, an increase in the size of individual lesions, and a gradual transition of the infiltrating cells from lymphocytes to cells of histiocytic type. Polymorphonuclear infiltration and vascular necrosis are present after the first day in very intense lesions in certain species, and focal necrosis may complicate extensive lesions. It is highly problematic, however, whether one may equate the demyelination produced in the zone of lymphocytic-histiocytic invasion in this disease with the destruction of bone and cartilage seen in adjuvant disease. In autoallergic encephalomyelitis as in adjuvant disease, clinical onset follows the initiation of lesion formation by one or more days.

Our findings focus attention on the role of mononuclear cells, specifically hematogenous cells with the morphologic aspect of medium lymphocytes, as the main agents of lesion formation. The nature of these cells and their pathogenic role appear to be the central problems confronting the student of cellular hypersensitivity at the present time.

SUMMARY AND CONCLUSIONS

Histologic examination of synovial, tendon, and connective tissue lesions in rats, sacrificed shortly before or shortly after the onset of adjuvant disease, showed that lesion formation is initiated by perivascular accumulation of mononuclear cells resembling medium lymphocytes. With increasing duration of disease, there is an increase in the number and size of individual lesions and a progressive increase in the number of cells of monocytic or histiocytic character in the infiltrates. At the same time, foci of necrosis appear in large or unusually intense lesions; and polymorphonuclear leukocytic infiltration occurs, both in necrotic zones and diffusely in many of the (pre-existing?) mononuclear lesions. These

changes were not observed in the earliest lesions studied and were never seen in the absence of the characteristic mononuclear cell infiltrates. Proliferation of fibroblasts, synoviocytes, and epidermis appear secondarily in areas involved by disease. Bone and cartilage destruction and pannus formation are late changes. Clinical abnormalities are apparent only one or more days after lesion formation has begun.

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