were performed with levamisole AMP activity was detected on brush border as well as on membranes of tubular invaginations, transport tubules and endocytotic vacuoles The basolateral labyrint in PT epithelium was not developed in contrary to metanephros (Narbaitz and Kacew 1978) The basolateral cell surfaces of mesonephric epithelium were projected in short interdigitating microvilli and expression of AP and AMP activities on their membranes suggested that this structural specialization should be involved in the transepithelial transport of PT

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Total and Local Changes in the Arthritis Adjuvans

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Abstract. Arthritis adjuvans was studied in the murine model An effect of different treatment (methotrexate, tauredon, collagen hydrolysate) was estimated in the course of developing disease (day 3, 5, 11 and 21) Repeated evaluation of body weight and peripheral blood leukograms as a total response of organism was performed Oedema of paw periarticular and tail regions, light- and electronmicroscopical screening and immunohistochemical investigation of prevalence of interleukin-1-β (IL-1β) and tumour necrosis factor (TNF-α) were estimated The most pronounced benefit effect of methotrexate at stabilization of the monocytes blood level, synovial membrane cell invasion and TNF-α immunopositivity was ascertained

Key words: Arthritis — Synoviocytes — Macrophages — Cytokines — Therapy

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Different kinds of arthritis represent serious complications for living circumstances of a sick organism Adjuvant arthritis (AA) is similar to reactive arthropathy, such as Reiter's syndrome, in which an inflammatory arthritis follows infection with no microbial invasion in the synovial space Decreased bone formation and increased resorption are indicated during the development of polyathritis in this known murine model which can serve as a comparable nosological unit for investigation of an effect of different pharmacotherapeutic trials and for evaluation of changes in the course of developing disease The aim of this study was to investigate an effect of some substances (methotrexate, tauredon, collagen hydrolysate) used for the therapy mainly of rheumatoid arthritis (RA) on the development of AA in rats

AA was induced by a single intradermal injection of Mycobacterium butyricum in Freund's complete solution (FCA) Development of AA was graded according to erythema and edema of paw periarticular and tail regions (Tab 3), body weight (Tab 2) was estimated simultaneously Animals were sacrificed at the day 3, 5, 11 and 21, blood plasma and samples from knee and elbogen joints of the paws were collected for morphological light- and electronmicroscopical analysis and immunohistochemical investigation of prevalence of IL-1β and TNF-α 72 female Lewis (LEW/Crl/CrlBr) rat were subdivided into four groups according to following treatment controls (C) – without any medication, collagen hydrolysate group (H) 0 1g/kg perorally five times a week, methotrexate group (M) perorally 0 1 mg/kg/week – and tauredon group (T) – intramuscularly 2 mg/kg/week – treatment Medication started on the first experimental day Samples for light microscopy were fixed with 4 % formaldehyde, decalcified and embedded by a common paraffin technique Specimens of prepared synovial membrane for electron microscopic evaluation were fixed in Karnovsky's mixture, dehydrated in ethanol and embedded in Epon 812 Semithin sections, stained by toluidine blue, were used for preparing of pyramids for ultrathin sectioning In sodium ethanolate deeponised semithin sections were processed by an indirect immunofluorescence method for demonstration of IL-1β and TNF-α Double stained ul trathin sections were evaluated in a JEM 100 B electron microscope ELISA methods were also used for determination of cytokines in blood plasma

Table 1. Percentage values of leukograms in investigated groups

	C3	C5	C11	M3	M5	M11	T3	T5	T11	H3	H5	H11
Neutro	27 3	53	56	20	47 7	53	20	52	56	24 3	43 3	52 7
± SD	8 20	4 1	0	0 8	9 4	2 2	11	2 2	0 9	4 7	4 5	2 1
Lymfo	58 3	36 3	36	68	38 7	35 3	67	38 3	33	63 7	46 7	36
± SD	9 5	6 2	2 4	2 4	11 1	2 9	9 6	1 2	4 8	4 7	2 4	4 5
Mono	13	9	8 3	11 3	12 7	11 3	12	8	11	12	9 3	10 3
± SD	2 2	2 2	2 4	3 6	0 9	1 9	1 6	1 4	2 9	0	2 5	3 3

The most pronounced changes at the day 3 concerned significantly increased values of lymphocytes (more than 60 %) in disadvantage to neutrophils in leukograms of all investigated subgroups without significant differences among investigated subgroups (Tab 1) Since day 5 the leukograms became of normal proportion with exception of the H subgroup where a higher prevalence of lymphocytes was prolonged up to the day 11 Body weight (Tab 2) increased up to the day 11, later was stabilized Starting day 11

Table 2. Body weight (in g)

day	0	5	10	13	17	20
C	113	143	151	143	137	137
M	117	140	152	150	141	143
T	119	144	152	146	143	144
H	121	143	148	146	136	142

Table 3. Hind paw thickening (mm)

day	0	5	10	13	17	20
C	5 9	5 9	6	6 4	8 3	8 5
M	5 9	6	6	7	8	9 5
T	6	6	6	7 5	8 4	9 4
H	6	6	6	7 4	9 1	9 3

increasing thickness of measured paws and tails could be established up to the day 21 (Tab 3) Only in the group M (methotrexate) further following thickening was observed On the contrary in the group T a decrease of thickening was estimated at the later stages of developing AA (between day 21 and 31)

A morphological appearance of non-altered surface of the synovial membrane was characterized by extremely flattened cell processes of synoviocytes, which represent non-continuos lining of joint cavity Beneath the surface numerous capillaries can be observed Cytoplasm matrix of fibroblasts, synoviocytes and endothelial cells have nearly identical electron density As a main morphological feature of early developing arthritis changes of synovial membrane were found A cytological activation of synoviocytes, together with their marked elevation presented almost continuous lining of synovial space Cytoplasm of synoviocytes contains formations of both types of endoplasmic reticulum as signs of a higher synthetic activity As a typical feature of some stromal fibroblasts a separation from fibrilar component of intercellular matrix can be found Their irregular thin processes fill translucent portion of intercellular matrix In the synovial loose connective tissue increased cellulization can be observed This cellular component consists predominantly of macrophages and relatively low portion of lymphocytes Some dendritic antigen presenting cells were identified electronmicroscopically Findings in the group M, where a cellular invasion was not so prominent, represent an exception In the group H only a higher participation of lymphocytes in loose connective tissue of synovial membrane stroma was detectable Findings of single mast cells belong to early changes in the synovial membrane in the experimental animals Pronounced vascularization of the superficial layer of synovial membrane was visible Stimulated surface layer of synoviocytes, frequent macrophages and lymphocytes and some large dendritic antigen presenting cells in the superficial stroma area were accompanied with a later experimental period except methotrexate trial only, as a markedly lower cell invasion into the synovial membrane was estimated To further findings in the intercellular matrix isolated free lipid droplets, originating from altered adipocytes, belonged Results of immunofluorescent methods of visualization of followed cytokines confirmed their localization predominantly in macrophages and dendritic cells but also activated synoviocytes were labelled by fluorescein of secondary antibody Some immunopositivity concerning TNF-α was detected on the collagen fibrilar component of synovial stroma, too ELISA method did not offer significantly different results in levels of estimated cytokines While TNF-α levels were determined regularly, IL-1β values were oft out of measurable range

In the last years the accumulated evidence incriminating various cytokines in the mechanism of synovial proliferation and joint destruction in rheumatoid arthritis was collected Especially IL-1β and TNF-α appear to directly contribute to tissue damage through induction of the release of tissue-damaging enzymes from synovial cells and articular chondrocytes (Arend and Dayer 1995), although many others cytokines are present in inflamed tissues There is a strong synergism between IL-1β and TNF-α production,

as was demonstrated in induction of the degradation of human articular cartilage during prolonged culture (Campbell *et al*, 1990) Intraarticular administration of TNF either prior to or after the induction of arthritis leads to an accelerated onset and more severe course On the other hand the administration of monoclonal antibodies against TNT leads to an attenuation of the severity of the inflammation and joint destruction Anti-TNT treatment may be primarily antiinflammatory but blocking IL-1β may be more effective in preventing cartilage destruction Suppression of adjuvant arthritis by oral administration of collagen not only type II, but also by types I and III was referred Unfortunately, cytokines did not determine in this association Histopathological examination of inflamed joint in AA revealed a massive infiltration of polymorphonuclear cells and synovial hyperplasia Cartilage and bone destruction is evident at later stage of disease Inflammatory conditions without involvement of lymphocytes are generally evident after only a few days, while specific immune reactions generally develop after 14 days Low-doses of methotrexate (MTX) belong to a standard second-line therapy for RA Last studies suggest that MTX inhibits IL-1β production (Segawa *et al*, 1997) The effect of MTX treatment on TNF production is more equivocal Gold compounds were used to inhibition of development of AA High concentrations of univalent gold inhibit IL-1β production by lipopolysaccharide-stimulated monocytes

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