Effect of testosterone on the kinetics of the development of suppressor cells in adjuvant arthritis

M. Tsukano, M. Kotani, K. Matsuno, K. Miyakawa and Y. Nawa[1,2]

Department of Anatomy, Kumamoto University Medical School, Kumamoto (Japan), 8 October 1984

Summary. The induction of unresponsiveness to mycobacterial adjuvant took a longer time in male DA rats than in female rats. A shift in the induction time of unresponsiveness in males toward the female type was brought about by castration, but could be reverted to the male type by the application of testosterone. The transfer study revealed that cells capable of preventing arthritis required a longer incubation time for their development in males than in females. This suggests that testosterone inhibits the development of suppressor cells in adjuvant arthritis.

Key words. Adjuvant arthritis; unresponsiveness; suppressor cells; sex difference; testosterone.

Adjuvant arthritis (AA) is readily induced in rats by an injection of mycobacterium in mineral oil, and is regarded as a cell-mediated immune response. Although human rheumatoid arthritis has a definite sex difference, with a greater prevalence of this disease in females, there is no settled view concerning the sex difference in the susceptibility to AA[3,4]. Unresponsiveness can be induced by pretreatment with subarthritogenic doses of mycobacterial adjuvant[5,6]. We found an interval difference between the onset of unresponsiveness in male and female rats.

Material and methods. Inbred DA rats, 8 weeks of age, were used. They were fed with standard laboratory chow and water ad libitum. Orchidectomy and ovariectomy were performed under ether anesthesia through a midline incision in the scrotal skin or through a bilateral flank incision. A discus pellet (6.8 mm diameter and 0.6 mm thickness) was made by pressing 40 mg testosterone powder (Merck, Darmstadt). The testosterone pellet was implanted into a small pocket of s.c. connective tissue made in the back skin between the scapulae. The serum testosterone concentration at the time of priming with the adjuvant was measured by radioimmunoassay in the Kitazato-Bristol Laboratories (Kanagawa). The results were found to be 589.2 ± 178.7 μg/dl in nontreated male rats, 19.7 ± 2.4 μg/dl in castrated male rats, and 2753.4 ± 368.8 μg/dl in castrated and pellet-implanted male rats. The testosterone level at the time of priming was also 62.7 ± 41.4 μg/dl in male rats, castrated, pellet-implanted, but with the pellet removed 3 days before priming.

The adjuvant was prepared by dispersing ground heat-killed *Mycobacterium tuberculosis* of a human strain Aoyama B (supplied through the courtesy of the Chemo-Sero-Therapeutic Research Institute, Kumamoto) in mineral oil (Paraffin flüssig Art 7161, Merck, Darmstadt) with a vortex mixer in a concentration of 0.0003–3.0 mg/ml. Each rat was injected intradermally with 0.1 ml of adjuvant into one hind footpad for the primary challenge. The second challenge was carried out intradermally at the base of the tail. The arthritis was evaluated by visual scoring of the joints according to the degree of swelling and redness. Scoring ranges were as follows: wrists, 0–5; ankles, 0–10; tarsi, 0–10; and each of the smaller joints (metatarsophalangeal and interphalangeal) 0–1, respectively. The lesions of the injected foot and/or tail were not included in the score. The maximum possible score was 40.

For cell transfer, the popliteal lymph nodes draining the injection site of the adjuvant were excised, minced, and gently squashed between the frosted ends of sterile microscope slides in a cooled Eagle's solution. The cell suspension was passed through two layers of surgical gauze to remove debris. The cells were washed three times and resuspended in the same fresh medium. The viability of the cells was more than 82% as determined by the trypan blue exclusion technique.

Results. DA rats showed a 100% incidence with a high severity of arthritis in the primary response to 0.03 or 0.3 mg mycobacterial adjuvant, as shown in figure 1. A dose of 0.03 μg or 0.003 mg adjuvant was subarthritogenic. No sex differences were found in the primary response.

In the secondary response to the arthritogenic dose of 0.3 mg adjuvant in rats primed with subarthritogenic 0.003 mg adjuvant, the longer the time interval between the primary and second challenges was, the lower the arthritic score became, and complete unresponsiveness was finally induced (fig. 2). When the induction time of unresponsiveness between the two sexes was compared, the male rats took a longer time (8 weeks) than the female rats (4 weeks). Orchidectomy 2 weeks prior to priming induced complete unresponsiveness at 4 weeks, as in females (fig. 3). In other words, the induction time of unresponsiveness in male rats shifted toward the female type by castration. Ovariectomy did not change the induction time. An application of the testosterone pellet at 2 days after orchidectomy up to the 7th day after priming could revert this induction time to the male type (fig. 3), whereas the pellet implanted up to 3 days before priming had no such effect.

Popliteal lymph node cells from donor rats at 4–16 weeks after receiving a subarthritogenic dose of 0.003 mg adjuvant were transfered i.v. into normal sex-matched recipient rats. Immediately after the cell transfer, the recipients were injected with the arthritogenic dose of 0.3 mg adjuvant into one hind footpad.

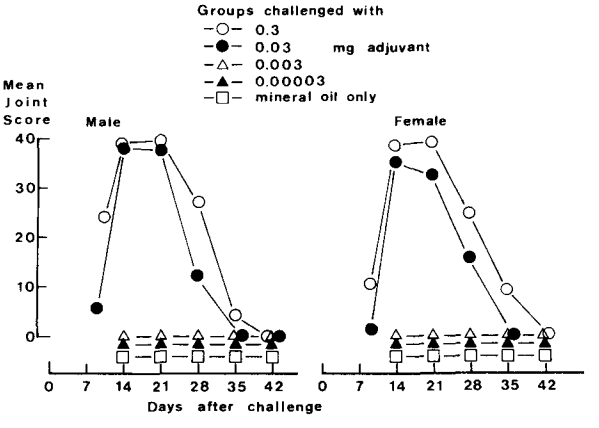

Figure 1. The clinical scores of arthritis in rats (21 males and 24 females) after receiving 0.00003–0.3 mg mycobacterial adjuvant. The control rats (16 males and 18 females) were injected with mineral oil only.

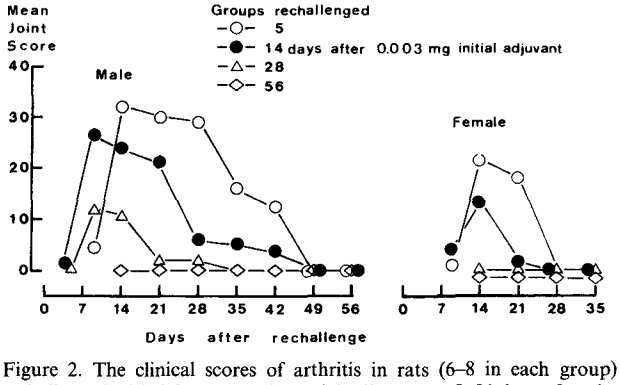

Figure 2. The clinical scores of arthritis in rats (6–8 in each group) rechallenged with 0.3 mg mycobacterial adjuvant at 5–56 days after the primary challenge with 0.003 mg of this adjuvant.

Complete prevention of arthritis was brought about by a transfer of 5×10^8 cells at 8 weeks in females and 16 weeks in males (table).

The maximum score of arthritis in recipient rats which received 0.3 mg mycobacterial adjuvant immediately after cell transfer. The cells were obtained from popliteal lymph nodes draining the injection site of 0.003 mg of this adjuvant 4–16 weeks before

Sex of donors	Number of cells transfered	Cells 4 weeks	8 weeks	16 weeks
Male	5×10^8		28.0 ± 5.7*(7/7)	0 (0/8)
	5×10^7		39.3 ± 0.8 (5/5)	25.2 ± 5.1 (4/4)
	0		37.1 ± 0.8 (4/4)	36.6 ± 0.5 (4/4)
Female	5×10^8	29.4 ± 5.6 (8/8)	0 (0/8)	
	5×10^7	38.4 ± 1.3 (5/5)	22.8 ± 2.3 (4/4)	
	0	37.8 ± 1.1 (5/5)	36.7 ± 1.0 (5/5)	

* Mean maximum joint score ± SE. ( ) Number of animal with arthritis.

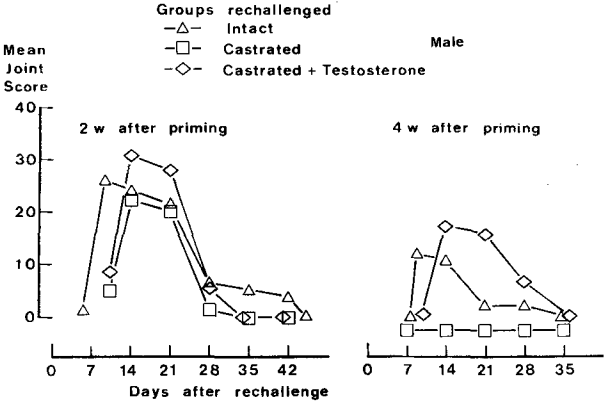

Figure 3. The clinical scores of arthritis in male rats (6–8 in each group) rechallenged with 0.3 mg mycobacterial adjuvant at 2–4 weeks after the primary challenge with 0.003 mg of this adjuvant. Castration was performed 2 weeks before priming. A testosterone pellet was implanted 2 days after castration and removed 7 days after priming.

Discussion. Unresponsiveness to mycobacterial adjuvant is due to an inhibitory response by suppressor cells, presumably T cells[4,5]. The present study indicated that male rats were more resistant than female rats in the induction of unresponsiveness in highly susceptible DA rats. The induction time of unresponsiveness in male rats shifted toward the female type after castration. An application of testosterone to castrated male rats could revert the induction time to the male type. Testosterone removed before priming had no such restorative effect. This suggests that testosterone acts suppressively on the kinetics of the development of suppressor cells. However, the definitive clarification of the mechanisms of testosterone action on suppressor cells must await further study. The transfer study also presented evidence indicating a sex difference in the incubation time for the development of suppressor cells. Although genetic control of the susceptibility to AA has been reported by other investigators[7,8], the present study suggests that testosterone could be a factor modulating the manifestation of this disease. Modulation of the expression of autoimmunity by androgen action on the thymic-dependent regulatory mechanisms has been reported in MRL/lpr and NZB × NZW F_1 mice [9,10].

1 Correspondence to Prof. M. Kotani, Department of Anatomy, Kumamoto University Medical School, 2-2-1 Honjo, Kumamoto 860, Japan.
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Covalent binding of aflatoxin B_1 to liver DNA in rats pretreated with ethanol

M. Marinovich[1,2] and W. K. Lutz[3]

Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, CH-8603 Schwerzenbach (Switzerland), 17 Juli 1984

Summary. Male Fischer F-344 rats were given ethanol in the drinking water and/or by single oral administration. Following this, the animals received p.o. 100 ng/kg of the hepatocarcinogen [^{3}H]aflatoxin B_1 (AFB_1). 24 h later, the level of DNA-bound AFB_1 was determined in the liver and was found not to be affected by any type of ethanol pretreatment. A cocarcinogenic effect of ethanol in the liver is therefore unlikely to be due to an effect on the metabolic activation and inactivation processes governing the formation of DNA-binding AFB_1 metabolites.
Key words. Carcinogenesis; DNA; covalent binding; aflatoxin; ethanol.

Although alcohol is listed as a factor contributing to between 2 and 4% of all human cancer deaths[4], pure ethanol has so far yielded negative results in animal tests for carcinogenicity (reported in Obe and Ristow[5]). Alcohol must therefore be considered to be a cofactor in the etiology of cancer[6]. One of the mechanisms postulated to explain its cocarcinogenic activity is the ability of ethanol to interact with drug-metabolizing enzyme systems involved in the activation of procarcinogens to reactive, DNA-binding intermediates. Enzyme activities can either be induced after repeated administration of ethanol or be inhibited by ethanol itself (see reviews by Lieber et al.[7] and McCoy et al.[8]). The susceptibility of the enzymes to this latter effect seems to be organ-specific, as it has recently been found that the first-pass clearance of dimethyl- or diethylnitrosamine[9] and of methylbenzylnitrosamine[10] is inhibited by ethanol in the liver but not in the oesophagus. These results might explain mechanistically some of the results describing a modulation of nitrosamine carcinogenesis by ethanol (Lieber et al.[7] McCoy et al.[11], Pour et al.[12]), including a shift of the target organ[13].

Since one of the target organs for the cocarcinogenic activity of