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SUPPRESSION OF EXPERIMENTAL ANTIGEN-INDUCED ARTHRITIS IN TRANSGENIC MICE PRODUCING HUMAN α-FETOPROTEIN

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SUMMARY: Experimental arthritis was induced in the knee joint of transgenic mice expressing human α-fetoprotein by immunization with methylated bovine serum albumin in Freund's complete adjuvant. In the control experiment with normal C57BL/6 mice, definite arthritis was observed in 55.6% (5/9) of the mice, but in only 21.1% (4/19) of the transgenic mice. This result suggested that human α-fetoprotein functioned as an immunosuppressant to ameliorate the development of the immune disease.

α-Fetoprotein (AFP), a major serum protein during fetal life, has been reported to have immunoregulatory functions in a variety of experimental systems although controversial reports have appeared (for review, see refs 1 and 2).

Of particular interests are reports that some experimentally induced autoimmune diseases in animals were inhibited by the administration of AFP. Putative autoimmune disorders in humans such as rheumatoid arthritis, myasthenia gravis, multiple sclerosis, systemic lupus erythematosus, etc. often show clinical remission in patients during the latter half of pregnancies (3) when the serum AFP is elevated to several hundreds ng/ml (1).

The development of experimental autoimmune myasthenia gravis in rabbits (4) and rats (5) has been reported to be prevented by the administration of human AFP. The same investigators also reported that human AFP suppressed experimental allergic encephalomyelitis in guinea pigs (6). However, others found no evidence that either rat or human AFP was able to ameliorate the

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Abbreviations: AFP, α-fetoprotein; mBSA, methylated bovine serum albumin; FCA, Freund's complete adjuvant; ELISA, enzyme-linked immunosorbent assay; TNF, tumor necrosis factor.

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development of allergic encephalomyelitis in rats (7). It is difficult to explain the discrepancies noted but may be due to that the protein was modified by the isolation procedures or due to non-AFP components in the preparations employed.

We have produced a transgenic mouse from C57BL/6 strain, designated TG-3, which expresses human AFP following genetic modification with AFP cDNA and a β-actin promoter/enhancer (8). In this TG-3 mouse, human AFP is produced ubiquitously by various tissues and the serum AFP concentration is constant, approximately 20 μg/ml, throughout the life. This value corresponds to the maternal mouse serum AFP level during the pregnancy and is more than 500 times higher than the serum level of normal adult mice (8). In experiments using this transgenic mouse, host defenses against bacterial infection with *Listeria monocytogenes* were observed to be decreased (9). Reduced production of cytokines detected at the early stage of the infection was suggested as the cause. This result indicated that human AFP in the transgenic animal plays an immunosuppressive role in the infection.

Induction of experimental arthritis in the knee joints of mice following the immunization with methylated bovine serum albumin (mBSA) with Freund's complete adjuvant (FCA) has been reported as a model of rheumatoid arthritis (10). In this study, the development of such arthritis in TG-3 transgenic mice which express human AFP has been explored .

MATERIALS AND METHODS

Induction of arthritis. Experimental antigen-induced arthritis was elicited in the knee joints of mice using mBSA (Sigma Chemical Company, St. Louis, MO). Male TG-3 mice at the age of 6-8 weeks were intraperitoneally injected with 100 μg of mBSA emulsified in 0.1 ml of FCA (H37Ra, Difco Laboratories, Detroit, MI). On day 7 after the start of the immunization, the same emulsion of mBSA in FCA was injected into two dermal flank sites. On day 21, 100 μg of mBSA in 10 μl saline was injected into the right knee joint. Contralateral knee joint was injected with 10 μl saline. Twenty-one days after the intra-articular injection, mice were sacrificed under ether anesthesia for histological examination. In control experiments, sex- and age-matched C57BL/6 mice were treated similarly.

Histological examination of arthritis. The terminated mice were perfused via the aorta with freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 and the knee joints removed. They were decalcified in 5% formic acid, processed, embedded in paraffin, sectioned, stained with haematoxylin and eosin, and subjected to microscopic examination. Severity of the arthritis was classified as mild and definite based on the following criteria: mild, minimal synovitis with occasional mononuclear cell infiltration; definite, clear hyperplasia of the synovium with marked mononuclear cell infiltration or severe synovitis with pannus and erosion of cartilage.

Serological analyses. Mouse serum obtained at the time of sacrifice was tested for AFP by radioimmunoassay (8) and for antibody against mBSA by ELISA which was a modification of that used to detect anti-tumor necrosis factor α(TNF-α) (11). Briefly, microtiter plates were coated with mBSA, blocked with skim milk and then incubated with 4-fold dilutions of serum up to 164,000. Bound IgG was detected by incubation with alkaline phosphatase-conjugated goat

anti-mouse IgG. The substrate we used was*p*-nitrophenyl phosphate and the product was measured by absorbance at 405 nm. Samples which showed more than 3 times higher absorbances than the background were judged positive.

RESULTS AND DISCUSSION

Histological evidence of arthritis was observed specifically in the right knee joint not only in the normal mice but also in the transgenic animals producing human AFP. However, the incidence was lower in the latter group, 42.1% (8/19), than in control mice, 77.8% (7/9), (χ^2 test, p=0.139). When the comparison was limited to definite arthritis, the difference became more marked, 21.1% (4/19) versus 55.6% (5/9), (p=0.077), respectively, and indicated that the disorder was less severe in transgenic than in control mouse group (Table 1).

Typical pathological features of the arthritis showed that many inflammatory cell infiltrations appeared perivascularly or diffusely and lining layers and synovium were hypertrophic with pannus formation (Figure 1A, B). No such findings were observed in the left knee joints injected with saline (Figure 1C) or in the right knee joints of transgenic mice which did not respond to the immunization (Figure 1D).

The human AFP concentrations in the sera of the transgenic mice were essentially the same, about 20 μg/ml, regardless of the development of arthritis. Antibodies against mBSA were detected in the sera of all mice immunized with mBSA. These titers showed considerable variations, 10,240 - 164,000, but differences between transgenic and normal mice or between mice with and without arthritis were not observed.

The implications of cytokines in the pathogenesis of the rheumatoid arthritis as well as its animal models have been reported. In experimental collagen-induced arthritis of mice,

TABLE 1

Incidence of development of arthritis

	Arthritis		
Mice	Mild	Definite	Total
Transgenic (19)	21.1% (4)	21.1% (4)	42.1% (8)
Normal (9)	22.2% (2)	55.6% (5)	77.8% (7)

The number of experimental animals is shown in parentheses.

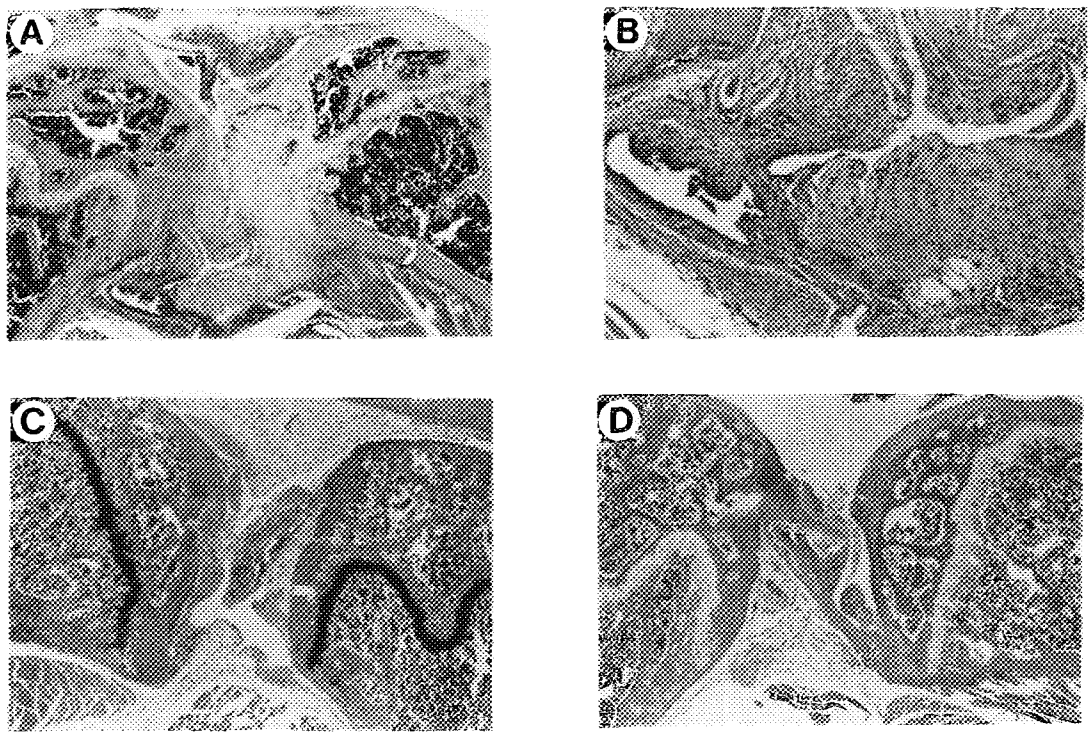

Figure 1. Histology of knee joints.
(A) In a non-transgenic mouse immunized with mBSA, severe arthritis is observed in the right knee joint (magnification ×35).
(B) High power (×85) view of the same region shows many infiltrated inflammatory cells and a hypertrophic synovium with pannus formation.
(C) The left knee joint of the same mouse injected with saline. No arthritis is observed(×35).
(D) The right knee joint of a transgenic mouse which did not respond to the immunization. No arthritis is observed(×35).

administration of antibody against TNF-α prior to the onset of the disease significantly reduced the severity of the disorder without reducing the incidence(11). Administration of TNF-α to animals with established disease was also effective in amelioration of the symptoms (11). It has been reported that interleukin 1, a cytokine itself, accelerates the development of murine experimental arthritis (12). The established disorder in the animal was exasperated by this cytokine (13). It is noteworthy that in bacterial infections, the production of TNF-α and interferon γ are reduced in TG-3 transgenic mice. This probably relates to the diminished early host defense(9). TNF-α is known to induce the production of interleukin 1 (11).

The results of this study strongly suggest that human AFP acts to suppress the experimental autoimmune disease. The possible mechanisms involved will require further

investigation. The TG-3 mouse will offer a simple and useful model system of high reproducibility in studies of such effects.

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