IMMUNOPATHOLOGICAL STUDY OF COLLAGEN-INDUCED ARTHRITIS IN F1 MICE

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Human rheumatoid arthritis (RA) is a chronic inflammatory disease with deformity and destruction of the articular cartilage and bone. The pathogenetic mechanism of chronic inflammation in RA is not known yet. However, it is said to be an autoimmune disease.

Collagen-induced arthritis (CA) induced with immunization of Bovine type II collagen(II) was reported in rat by Trentham and in mice by Coutenay) and it is considered to be a good experimental model of RA.

The arthritis in CA is frequently observed in DBA/1J male mice (DBA) which have H-2q haplotype. However, polyarthritis in the BALB/c female mice (BALB) with H-2d haplotype due to intra-abdominal sensitization with heat-killed E.coli 0:14 was induced by Aoki et al, who reported that proliferative synovitis with pannus formation could be seen2). Therefore, in this paper we prepared F1 mouse siblings (BDF1) between BALB and DBA to induce arthritis and described interesting results.

MATERIALS AND METHODS

BALB, DBA and BDF1 (BDF1 male, BDF1 female) were used for experiments at 8-9 weeks. Antigen of II was purchased from Collagen Institute. Mice had 100ug of II in the form of emulsion with Freund’s complete adjuvant injected intracutaneously into the tail base. 3 weeks later, collagen alone was injected into the tail base. Each mouse after sacrifice was fixed with formalin, decalcified by ethylenediaminetetraacetic acid and embedded in paraffin. HE, toluidine blue metachromasia (pH4.2) and Safranin O staining were carried out and indirect immunostainings of IgG, complement (C3), IL-1 and chondroitin sulfate were done by the avidin-biotin-peroxidase complex method. The mice sera were collected from the orbital veins after regular intervals and kept at -80°C until use. For IgG subclasses and IgM anti-collagen antibodies titers, the ELISA method was used.

RESULTS AND DISCUSSIONS

In the DBA group, IgG1, IgG2a and IgG2b anti-collagen antibody levels were at a comparatively high titer. In BDF1 female group, further enhancement of the antibodies was seen though each mouse was slightly different. Concerning the histological findings on CA, the arthritis in red and swollen limbs in three groups was observed on the 33rd day after sensitization. There were some differences in redness and swelling among the three groups. In the DBA group, remarkable infiltration of polymorphonuclear leukocytes (PMN) and mononuclear cells (MNC) can be seen. We can also observe debris and PMN in joint spaces. The surface of the cartilage became thin and destruction of the bone was observed. The synovial proliferations and infiltration of MNC and osteoclasts in the articular tissue can be seen. CA was also found in both the elbow and knee joints. In the BDF1 male group, the proliferation of synovium accompanied by infiltration of MNC, pannus formation and the cartilage with rough surface were also observed. During progression, capillary vessels and infiltration of macrophages and PMN were commonly observed. In the BDF1 female group, arthritis was more remarkable than in the others (Fig.1). CA was found in the larger joints, including both knees and elbow joints. In order to study the changes in cartilage, toluidine blue, Safranin O stain
and chondroitin sulfate immunostain were done but the degrees of staining in DBA, BDF1 male and BDF1 female (Fig.2) were less, suggesting that proteoglycan was released from the cartilage. Immunological localization of IgG and C3 was seen not only in the cartilage and the surface layers of synovial membranes but also within the infiltrated PMN of the joint space from the BDF1 female group (Fig.3a,3b). Staining results for IL-1 in the BDF1 female group (Fig.4) were almost the same as those for IgG and C3. It was of note that BDF1 had stronger arthritis, especially in female mice, and it also showed arthritis at the larger joints such as both knees, elbows and shoulders. Furthermore, anti-collagen antibody levels were higher in IgG1, IgG2a and IgG2b and BDF1 female group had a higher titer than the others. The present study suggested that the BDF1 female mouse is a good model with higher responsiveness to CA as a result of the study indicating that BDF1 female mouse has both higher anti-collagen antibody levels and a higher incidence of arthritis.

Fig.1: Severe arthritis in BDF1 female (H.E.)

Fig.2: Faint staining with chondroitin sulfate (Immunostain)

Fig.3a,3b: Immunological localization of IgG(3a) and C3(3b)

Fig.4: Immunological localization of IL-1

REFERENCES