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Acute Inflammatory Response in Experimental Skin Lesions of Patients with Ankylosing Spondylitis†

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Two different stimuli, tetanus toxoid (TT) and autologous fibrin (AF), were used to investigate the inflammatory response in ten patients with ankylosing spondylitis (AS); in seven persons who had a family history of AS and were HLA-B27 positive but had no clinical signs of AS; and in eight nonarthritic controls. The purpose was to determine whether the leukocytic response in an experimental Rebuck skin window lesion was the same or different from that seen in the synovial fluid of AS patients when in vivo-formed fibrin flakes were present. Four lesions were produced on each subject, with TT added to one and AF applied to the other three. The TT-stimulated lesion and one AF lesion were serially sampled after 6, 12, and 24 hours. The other two AF lesions were sampled only once at either 12 or 24 hours. Cells adhering to the coverglass surface were stained with Leishman's stain. A differential count of 500 leukocytes was performed on each of the 192 samples.

Our data suggest that exudative neutrophils do not recycle normally either in the experimental skin lesion of AS patients or in asymptomatic but clinically normal kindred of AS patients who are HLA-B27 positive. These findings agree with our previous in vivo observation that persistent fibrin flakes in the synovia of AS patients are associated with low numbers of exudative neutrophils. Diagnostic application of the Rebuck skin window lesion to determine the neutrophil response for ankylosing spondylitis may depend on the inflammatory response not following the HLA-B27 genetic marker.

In vivo formed fibrin, which persists in some arthritic joints (1), has been noted especially in patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS), where fibrin flakes are an obvious constituent of the aspirated exudative synovial fluid (Fig. 1). In RA, its persistence in the synovia correlates with an increase in the amount of synovial neutrophil emigration (2,3). By contrast, in the peripheral joints of AS patients, it is associated with a paucity of synovial exudative neutrophils and a significantly lower total synovial leukocyte count (4).

This study was designed to investigate the inflammatory dynamics in a Rebuck skin window using an antigenic and a nonantigenic stimulant in nonarthritic healthy controls, patients with classical AS, and in asymptomatic, normal persons who were HLA-B27 positive and had a family history of AS. The purpose was to determine whether the leukocytic emigration in an experimental skin lesion was the same or different from that seen in the synovial fluid of AS patients when in vivo formed fibrin flakes were present.

Materials and Methods

Ten patients were selected with radiological evidence of bilateral sacroiliitis and paravertebral ligament ossification. Each also exhibited clinical signs and symptoms of AS, but had no signs of any of the other seronegative spondyloarthritic diseases. Eight persons served as controls and were matched by age and sex. Similarly, seven persons (hereafter referred to as HLA-B27 kindred) were chosen who were HLA-B27 positive, were clinically free of AS, and were siblings of a parent with AS.

The volar surface of the forearms was chosen for the site of the skin lesion. A sterile No. 22 Bard Parker Blade was used, and with aseptic technique the epithelium was scraped from an area about 4 mm in diameter. The presence of the fine bleeding points indicated when the proper depth had been reached. Four such lesions, two on each forearm, were prepared on each person in this study. To one lesion, a drop of the antigenic stimulant, tetanus toxoid

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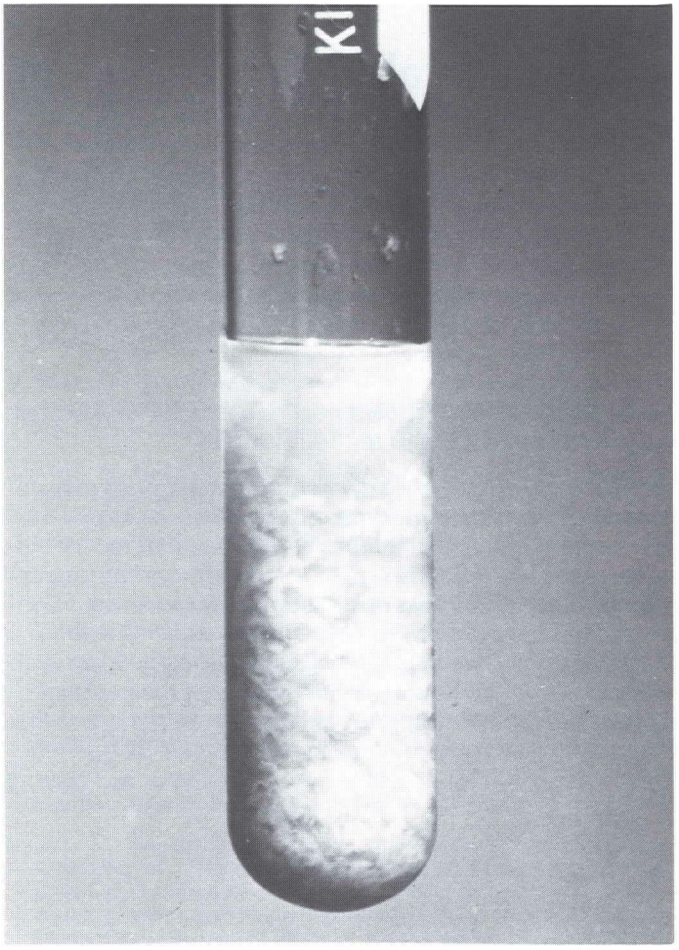

Fig. 1
Specimen of synovial fluid filled with fibrin flakes formed in vivo; fluid was aspirated from the joint of a patient with chronic RA.

(TT), was added. The lesion was surmounted with a sterile glass coverslip protected by a cardboard that was held in place by adhesive tape. After six hours, the coverglass was removed and replaced with another sterile coverslip. The same procedure was repeated at 12 and 24 hours, after which time sampling was terminated. Exudative leukocytes adhere as a monocellular layer on the surface of the coverslip. After the lesion had been removed, the cellular population on the coverglass was air dried, fixed, and stained with Leishman's stain.

The other three lesions were similarly prepared except that the stimulant applied was autologous fibrin (AF), which was prepared with sterile technique. Blood was drawn from the patient for a complete blood count and another 9 ml of blood obtained and mixed with 1 ml of sterile 3.8% sodium citrate. Plasma was removed from the anticoagulated specimen after centrifugation for 5 minutes. For each skin lesion stimulated with AF, 0.25 ml of plasma and 0.75 ml of sterile saline were mixed together, approximately 30 units of bovine thrombin was added, and the mixture was then slightly agitated. The gelatinous precipitate of AF forms on the surface of the coverslip which is subsequently inverted and placed in contact with the skin lesion. This second window was sampled at 6, 12, and 24-hour intervals, as described previously, but the third skin lesion was sampled only after 12 hours and then terminated. The fourth skin lesion, also AF-stimulated, was sampled only after 24 hours and then terminated. These specimens were stained and prepared for viewing with light microscopy by being permanently mounted on a glass microscope slide. For each coverglass specimen, five random areas were selected for cell differential counting, and 500 exudative leukocytes per sample were evaluated to determine the percentages of cell types. A differential cell count was performed on each of the 192 samples.

The method applied to test statistical reliability was the multivariate and univariate one-way ANOVA and the two sample student t test, one-tailed.

Results

The data for the TT-stimulated skin lesions are summarized in Fig. 2 for the control group, for AS patients, and for the HLA-B27 kindred. The percentage of neutrophil emigration of the control group followed a previously observed pattern

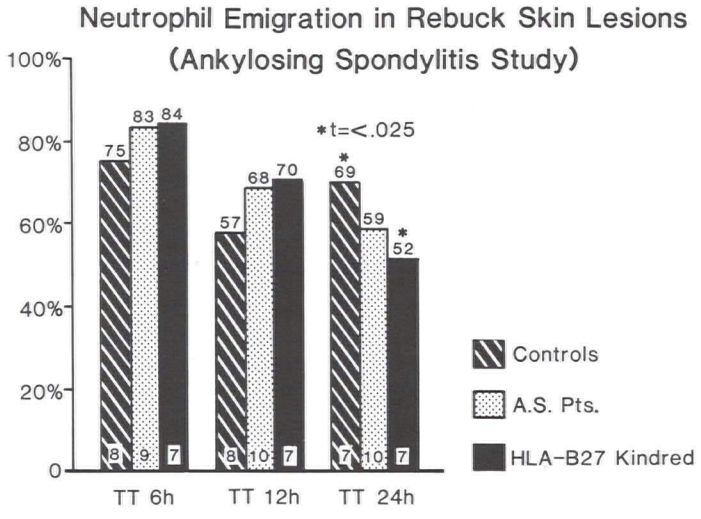

Fig. 2
Neutrophil response (%) to TT stimulation at 6, 12, and 24 hrs in the three groups studied. At 6- and 12-hr intervals, there were no essential differences between groups, but at 24 hrs neutrophil emigration between healthy controls and HLA-B27 kindred (P = 0.25 student t one-tailed test) was significantly reduced. Numbers at the base of the columns refer both to the number of patients included and to the number of individual samples analyzed at that particular hour.

for antigenic stimulation at the 6-hour, 12-hour, and 24-hour intervals. No significant difference was discovered between the three groups at the 6- and 12-hour intervals, but, at the 24-hour interval, the mean neutrophil emigration was significantly reduced to 52% in the HLA-B27 kindred when compared to the control mean of 69% (P=0.025 student t one tail test). A similar trend was observed between the AS patients, who had a mean percentage of neutrophil emigration of 59%, and the controls (P=0.039).

The data for AF-stimulated skin lesions are summarized in Fig. 3. The data for the 12- and 24-hour interval specimens contain two samples each, because no significant differences were found in the percentage of cellular emigration observed whether AF was applied only initially or for 12 or 24 hours.

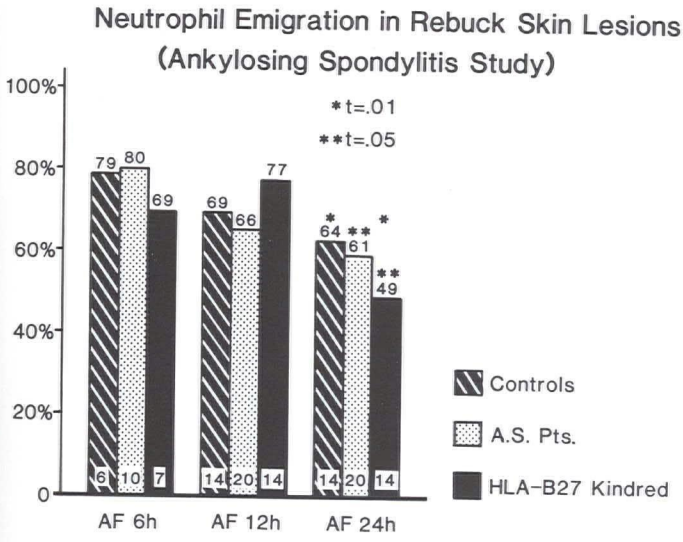

Fig. 3

Neutrophil emigrations to AF were similar for all three groups at 6- and 12-hr intervals, but at the 24-hr interval they were significantly decreased between controls and HLA-B27 kindred (P = 0.01). In addition, HLA-B27 kindred show a significantly decreased neutrophil response to AF at the 24-hr interval when compared to the AS patients (P = 0.05 student t one-tailed test). Numbers in the base of the columns at the 6-hr interval refer both to the number of patients included and the number of samples analyzed, but the 12-hr and 24-hr numbers refer only to the number of samples, which were 2 per patient, as described in the text.

Again, neutrophil emigration was essentially the same for the three groups at 6- and 12-hour intervals, but at 24 hours the mean neutrophil emigration for the HLA-B27 kindred was significantly less than for the control group (P=0.01). In addition, at the 24-hour interval, the amount of neutrophil emigration for the HLA-B27 kindred decreased to 49%, while the AS patients had a mean of 61% (P=0.05).

Although the percentage of neutrophilic leukocytes that emigrated differed significantly at certain times, the morphology of the exudative neutrophils was similar for all groups. In the three groups studied, the morphological cellular response was similar with TT or AF stimulation of the skin lesion at both the 6-hour interval (Fig. 4) and at 12 hours. However, at the 24-hour interval, the cellular response to both TT and AF for the HLA-B27 kindred was typified by an increased percentage of mononuclear leukocytes with a consistent decrease in neutrophil emigration when compared to controls. One of the differences noted in this group was that there were some eosinophils in the AF-stimulated skin lesion at 24 hours that were not seen in the TT-stimulated lesion at that hour (Fig. 5).

Discussion

Fibrin formation is a known component of the inflammatory process. Fibrinopeptides and fibrin degradation products are recognized as major mediators of inflammation derived from the plasma clotting system. Although fibrin related material does not initiate joint disease, its persistence in a joint contributes to a sustained neutrophil emigration in RA (3). When the skin lesion technique of Rebuck was used with a group of RA patients to determine the neutrophil response to AF, it was found that while the neutrophil response to AF was generally increased, the TT antigen stimulation in those patients produced a normal neutrophil emigration (6).

However, the data derived from our study differ from those results. Our AS patients failed to show the normal recycling of neutrophils at the 24-hour interval, and the mean percentage of neutrophils to TT was significantly decreased (P=0.039). In addition, the neutrophil emigration to TT was decreased at 24 hours in the HLA-B27 kindred when compared to the healthy controls (P=0.025). Why the reduced neutrophil response should occur in AS patients and their kindred, while a normal response to antigenic stimulation was found in RA patients is unclear. In general, the immune response of AS patients has been considered normal and that of RA patients overactive or exaggerated.

The mean neutrophil emigration to AF also differed in AS patients, although it was found to be the same as in nonarthritic controls. Others have reported that in the RA patient neutrophil emigration to AF is intensified (6). However, the neutrophil emigration observed in the HLA-B27 kindred was reduced significantly when compared to the normal response (P=0.01) and to the AS patients (P=0.05). This unexpected finding might be predictive of future disease, although such persons will need longitudinal study to detect signs of AS. Another explanation for the reduced neutrophil response is an association with the genetic marker HLA-B27. Other HLA-B27 positive, seronegative spondyloarthritic patients with psoriatic ar-

thritis and Reiter's syndrome need to be investigated, since such data might provide the evidence to link the reduced neutrophil response to TT and AF observed in AS kindred with HLA-B27 to the B-27 locus.

Fibrin flakes in RA patients recycle neutrophils rich in lysosomal enzymes. These enzymes not only contain alkaline proteases that can resolve fibrin but acid proteases that can destroy cartilage and bone (7). However, the persistence of fibrin formation without the usual neutrophil emigration might contribute directly or indirectly to the ultimate pathological feature of AS, which is a fibrous and bony ankylosis of spinal articulations.

Acknowledgments

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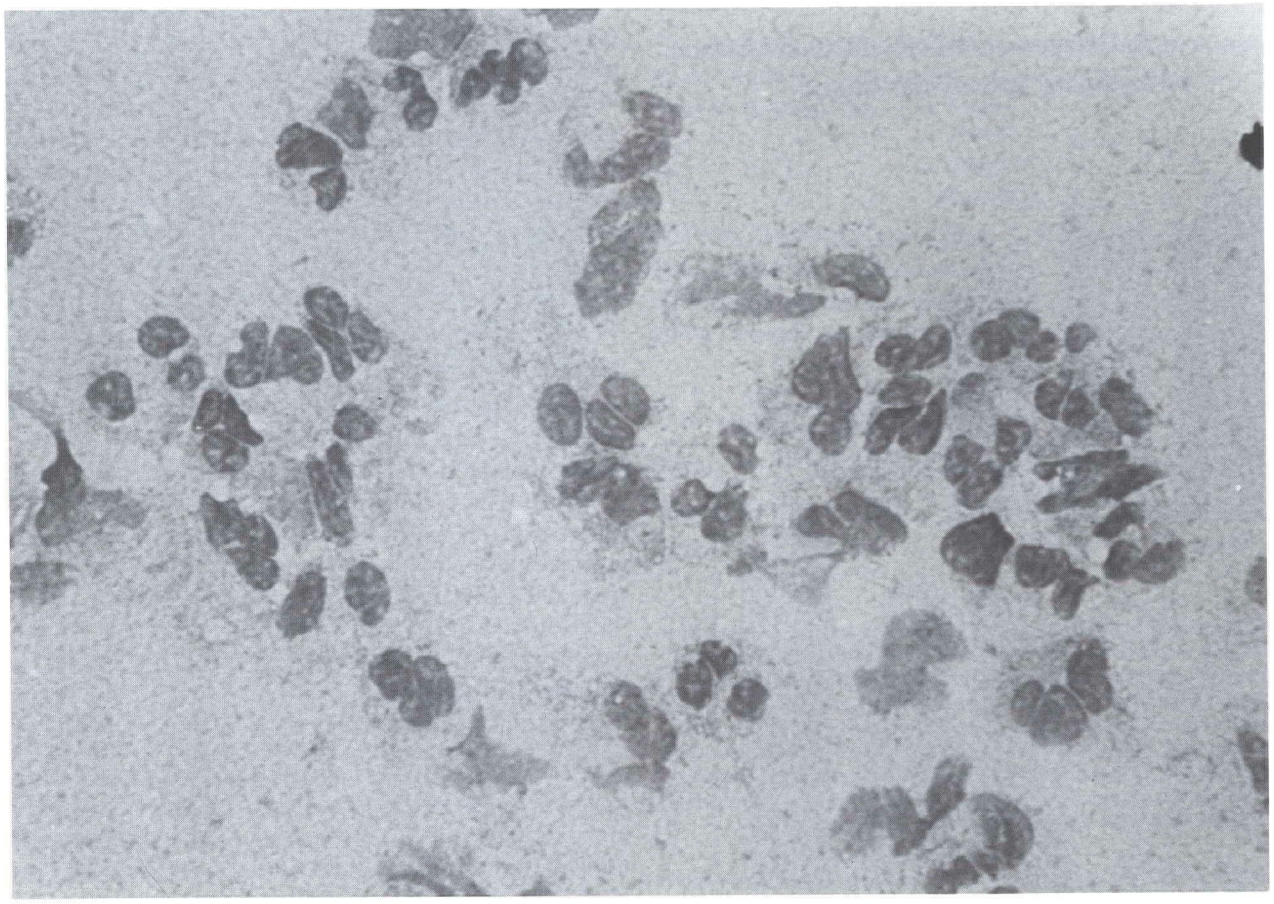

Fig. 4

In normal individuals, acute cellular inflammation 6 hrs after the addition of either TT- or AF-stimulated skin lesions exhibits primarily neutrophil emigration. AS patients and HLA-B27 kindred had a similar response (X1000).

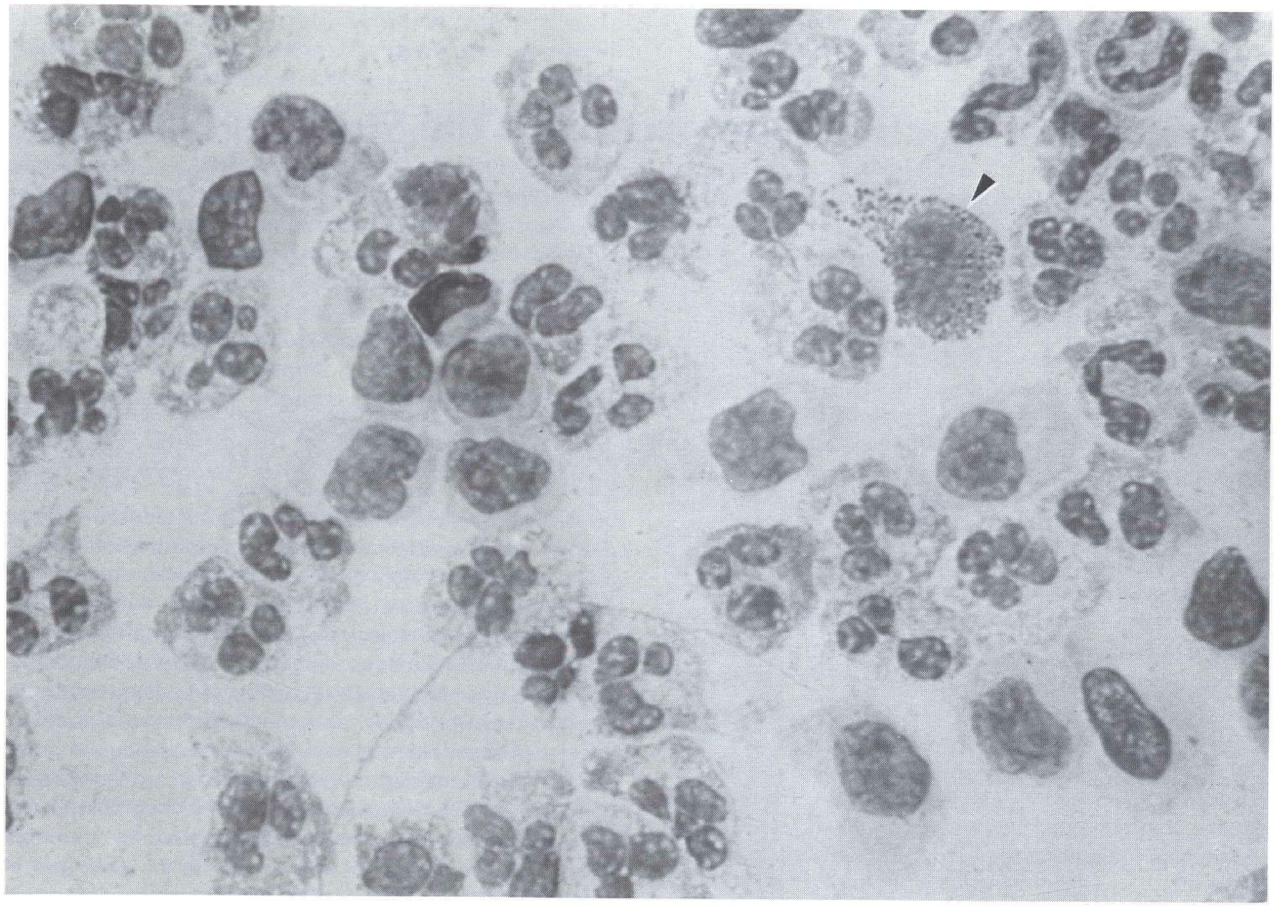

Fig. 5
Typical exudative leukocyte emigration pattern at the 24-hr interval in HLA-B27 kindred to TT or AF stimulation of the skin lesion. Eosinophil (arrow) was seen at the 24-hr interval with AF stimulation (Leishman's stain, X1000).

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