Enhanced neutrophil migration in vivo HLA B27 positive subjects

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SUMMARY Chemotaxis of polymorphonuclear leucocytes in vivo was studied in patients with previous yersinia arthritis and in healthy subjects with or without HLA B27 by means of a skin chamber technique. Irrespective of previous arthritis the number of neutrophils in the chamber media was significantly higher in HLA B27 positive subjects than in those without HLA B27. The amounts of prostaglandins E_2 , $F_{2\alpha}$, and 6-keto- $F_{1\alpha}$ in the chamber media correlated positively with the corresponding cell counts. The present results give credence to the view that the hyperreactive neutrophils and the vasodilatory prostaglandins produced by them can together trigger a vicious circle which results in increased inflammatory symptoms in patients with yersinia arthritis who have HLA B27 as compared with those who lack this antigen.

Reactive arthritis is a form of nonpurulent joint inflammation which follows an infection elsewhere in the body. In addition to rheumatic fever, a sequela of beta-haemolytic streptococcal pharyngitis, reactive arthritis can be triggered by enteric or urogenital infections, such as *Yersinia enterocolitica*, salmonella, shigella, chlamydia, or campylobacter infections.^{1 2} Reactive enteroarthritis is associated with the histocompatibility antigen HLA B27, about 80% of the patients having this antigen.¹

In HLA B27 positive patients the inflammatory symptoms, such as arthritis, ocular inflammation, and urethritis, are more prolonged and more frequent, and the inflammatory signs heavier, than in HLA B27 negative patients with yersinia arthritis.³ The reason for this difference is unknown. However, the findings that polymorphonuclear leucocytes from HLA B27 positive subjects, irrespective of yersinia arthritis, show high chemotaxis in vitro,⁴ and that sera from these subjects stimulate neutrophil migration in vitro more than sera from HLA B27 negative subjects,⁵ suggest that high phagocyte activity may contribute to the inflammatory symptoms in HLA B27 positive patients. To find out whether the in vitro findings are relevant to the in-vivo conditions

neutrophil chemotaxis in HLA B27 positive and negative subjects was tested by means of a skin chamber technique. We also analysed the concentrations of prostaglandins E_2 , $F_{2\alpha}$, and 6-keto- $F_{1\alpha}$ in the chamber media to evaluate the role of these arachidonic acid metabolites in the enhanced neutrophil migration.

Subjects and methods

Subjects. Twenty HLA B27 positive subjects (11 women and 9 men, mean age 38 years, range 28–51) and 20 HLA B27 negative subjects (17 women and 3 men, mean age 34 years, range 22–46) were studied. Fourteen of the HLA B27 positive subjects and 3 of the HLA B27 negative subjects had had yersinia arthritis 2–11 years earlier (mean 6 years). The patients were selected from those in the follow-up study.³ They had radiologically normal sacroiliac joints and no joint symptoms. None of the subjects studied had any signs of infection or inflammation or received any medication at the time of the skin chamber test.

Skin chamber test. Plastic chambers designed for assessing leucocyte migration in vivo⁶ were obtained from Sandoz Ltd (Basel, Switzerland). Four to 8 blisters were induced on the lower abdominal skin by suction for 60–90 minutes by a device described in

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detail.⁷ Blister roofs were cut off. The chambers (capacity 0.8 ml) were then firmly taped over the skin windows and filled with fresh 50% autologous serum in Hanks's balanced salt solution. After 24 hours the chambers were emptied and removed. Each skin lesion was covered with transparent film, delineated, and the area measured by means of a projection microscope and planimeter. Aliquots of 0.7 ml of pooled chamber fluid supernatants of 16 HLA B27 positive and 17 HLA B27 negative subjects were mixed with 70% ethanol and stored at -20°C until the analysis of prostaglandins.

Cell counts. Blood samples for white cell counts were taken immediately before the start of the blistering process. The total number of cells in each chamber was counted and expressed as cells per cm^2 of skin lesion area, and the mean of replicate chambers was determined.

Prostaglandin analyses. After extraction of the samples with Amberlite XAD-2 (BDH Chemicals Ltd, Poole, England) prostaglandins E_2 , $\text{F}_{2\alpha}$, and 6-keto- $\text{F}_{1\alpha}$ were measured by specific radioimmunoassays based on (^3H) prostaglandin (New England Nuclear, Boston, Massachusetts, USA), and dextran-charcoal separation of the antibody-bound and free fractions. Antibody, 0.1 ml, plus 0.1 ml of standard solution and 0.1 ml of (^3H) prostaglandin (15 000 d.p.m.) were used. The incubation time was 1 hour at room temperature.

The specificities of prostaglandin $\text{F}_{2\alpha}$ and 6-keto- $\text{F}_{1\alpha}$ antibodies prepared by us were tested against other prostaglandins. All cross-reactions were less than 1% at the 50% displacement level. Prostaglandin E_2 antiserum was purchased from The Pasteur Institute (Paris, France). The recovery of tritiated prostaglandin tested by these radioimmunoassays was 88–109% ($n = 15$) and the coefficients of intra- and interassay variations were 6.5–8.9% and 9.1–14.9%, respectively.

Statistical treatment. Geometric means and Student's t test were used in comparisons between HLA B27 positive and negative subjects and in comparisons between subjects with and without a history of yersinia arthritis. When the subjects were further analysed on the basis of both HLA B27 and previous arthritis, the Mann-Whitney U test was used as follows: HLA B27 positive healthy subjects versus HLA B27 negative healthy subjects, HLA B27 positive subjects with previous yersinia arthritis versus HLA B27 negative subjects with previous arthritis, HLA B27 positive healthy subjects versus HLA B27 positive subjects with previous arthritis, and HLA B27 negative healthy subjects versus HLA B27 negative subjects with previous arthritis.

The correlations between the total cell count of the replicate skin chambers and the total amounts of

prostaglandins E_2 , $\text{F}_{2\alpha}$, and 6-keto- $\text{F}_{1\alpha}$ in the corresponding chamber media were evaluated by Pearson's correlation coefficient. Because in one subject the prostaglandin E_2 value was aberrant and seemed to increase the chance of positive correlation (Fig. 3), the Spearman rank correlation test was also performed for prostaglandin E_2 .

Results

The mean number of cells per cm^2 in the skin

Fig. 1 Mean leucocyte counts in the skin chambers in HLA B27 positive and negative subjects. Healthy subjects (○); subjects with previous yersinia arthritis (●); geometric mean $\pm$ SE (—+—).

chamber was higher in 20 HLA B27 positive subjects than in 20 HLA B27 negative subjects (Fig. 1). The difference was small but statistically significant. The proportions of polymorphonuclear leucocytes in the chamber media in the 2 groups were identical, $97.8 \pm 0.2\%$ (mean $\pm$ SE). The corresponding erythrocyte counts per 200 leucocytes were 0.5 ± 0.1 and 0.7 ± 0.2 .

In peripheral blood, leucocyte counts in the 2 groups were $5.9 \pm 0.3 \times 10^9/l$ and 6.1 ± 0.3 , and neutrophil counts were identical, 3.2 ± 0.2 . In the skin chamber technique the number of exuding leucocytes correlated positively with the area of the skin lesion.⁸ The average area of the skin lesion in HLA B27 positive subjects (0.22 ± 0.01 cm²) was the same as that in HLA B27 negative subjects (0.23 ± 0.01 cm²). Because the subject groups studied included both healthy individuals and those with previous yersinia arthritis the results were further analysed.

In healthy subjects the mean number of cells per

cm² was significantly higher in the HLA B27 positive group ($n = 6$, Fig. 1) than in the HLA B27 negative one ($n = 17$, $p < 0.02$). When the results shown in Fig. 1 were combined on the basis of previous arthritis, the difference between the subjects with previous arthritis ($n = 17$) and those without ($n = 23$) was not significant. Neither within the HLA B27 positive nor within the HLA B27 negative group was the difference between healthy subjects and those with previous arthritis significant.

The mean concentrations of prostaglandins E₂, F_{2 α} , and 6-keto-F_{1 α} were higher in the HLA B27 positive group than in the HLA B27 negative group, but the differences were not significant (Fig. 2). There was a positive correlation between the total number of leucocytes in the 4 to 8 skin chambers and the amounts of the 3 prostaglandins in the corresponding chamber media (Fig. 3). The differences in the mean prostaglandin concentrations between healthy subjects and those with previous yersinia arthritis were not significant.

Fig. 2 Mean prostaglandin (PG) concentration in the chamber media in HLA B27 positive and negative subjects. Healthy subjects (○); subjects with previous yersinia arthritis (●); geometric mean $\pm$ SE (—+—).

Fig. 3 Correlations between total chamber cell count and prostaglandin (PG) amounts. Pearson correlation coefficients are shown, for PGE₂ Spearman rank correlation coefficient $r_s = 0.48$, $p < 0.01$.

Discussion

According to the 2-component hypothesis⁹ the generation of acute inflammatory oedema depends on both local vasodilatation and increased microvascular permeability. There is evidence that polymorphonuclear leucocytes have an important part in the control of these vascular changes.^{10, 11} Neutrophils accumulate at the site of inflammation in response to chemotactic signals, phagocytose foreign invaders and damaged tissue components, and release lysosomal enzymes. When exposed to chemotactic peptides in vitro, polymorphonuclear leucocytes also release prostaglandins, including those of type E with vasodilatory activity.¹² Furthermore, in penetrating through the capillary walls they increase vascular permeability by a yet undetermined mechanism.¹¹ Recently the 2-component hypothesis has also been shown to be applicable to man by using bradykinin to increase vascular permeability and prostaglandin E₂ to induce hyperaemia.¹³

The results in the present study indicate that neutrophils accumulate in the skin chamber in response to 50% autologous serum in greater numbers in HLA B27 positive subjects than in HLA B27 negative subjects. The phenomenon was associated with HLA B27 but not with previous yersinia arthritis. The results are in good accordance with the in-vitro findings that, irrespective of previous yersinia arthritis, both the chemotactic responses of polymorphonuclear leucocytes and the chemokinetic

activities of sera from HLA B27 positive subjects are higher than those from HLA B27 negative subjects.^{4, 5} Because we studied in vivo chemotaxis using fresh autologous serum, it was not possible to determine whether the cells or the sera, or both, were responsible for the enhanced responses.

The mean areas of the skin lesions and the mean numbers of neutrophils in peripheral blood in HLA B27 positive and negative subjects were much the same. Furthermore, on the basis of both visual inspection of the blister grounds and erythrocyte counts in the chamber media, bleeding, if any, was negligible. Thus these variables cannot explain the difference observed between the 2 groups.

The number of cells in the replicate skin chambers, 98% of the cells being polymorphonuclear leucocytes, correlated positively with the amounts of prostaglandins E₂, F₂α, and 6-keto-F₁α in the corresponding chamber media. Human polymorphonuclear leucocytes can release prostaglandins E₂ and F₂α.¹⁴ Prostacyclin, the unstable parent compound of 6-keto-F₁α, is known to be a product of vascular endothelium,¹⁵ and thus 6-keto-prostaglandin-F₁α in the chamber media may have originated from skin capillaries stimulated to release prostacyclin by the suction procedure.¹⁶ Other possible sources of 6-keto-prostaglandin-F₁α are the few mononuclear cells¹² in the chamber media and macrophages¹⁷ in the blister grounds.

On the basis of the results we suggest that in HLA B27 positive subjects, as compared to HLA B27

negative ones, hyperresponder neutrophils in highly chemokinetic surroundings accumulate at the site of inflammation excessively. They liberate in concert substantial amounts of vasodilatory prostaglandins which cause hyperaemia. Increased blood flow brings additional neutrophils to exude and release vasoactive prostaglandins. Although this hypothesis is based on results obtained by using a skin chamber technique, it is apparent that such a vicious circle can also function elsewhere in the body, and consequently it can explain the more severe inflammatory symptoms in HLA B27 positive patients with yersinia arthritis as compared with HLA B27 negative patients. Furthermore, we consider that this model may have wider implications. Because the hyper-responsiveness of the polymorphonuclear leucocytes was associated with HLA B27 and not with previous yersinia arthritis, the self-perpetuating cycle suggested may play a role in the pathogenesis of other inflammatory diseases linked with HLA B27, such as ankylosing spondylitis and Reiter's disease.

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