Epidemiol. Infect. (1994), **112**, 367–373

Host-microflora interaction in Systemic Lupus Erythematosus (SLE): colonization resistance of the indigenous bacteria of the intestinal tract

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(*Accepted 14 October 1993*)

SUMMARY

Experimental data suggest a role for the microflora in Systemic Lupus Erythematosus (SLE). Anti-ds-DNA antibodies may be pathogenic in SLE by forming immune complexes with DNA. Foreign bacteria in the intestines could constitute the stimulus for anti-ds-DNA antibody production in SLE. Colonization Resistance (CR) is the defence capacity of the indigenous microflora against colonization of the intestines by foreign bacteria. A low CR implies increase of translocation of bacteria and a higher chance of subsequent, possibly DNA-cross-reacting antibacterial antibody production.

We measured CR by a comprehensive biotyping technique in healthy individuals and patients with inactive and active SLE. CR tended to be lower in active SLE patients than in healthy individuals ($P = 0{\cdot}09$, Wilcoxon one sided, with correction for ties). This could indicate that in SLE more and different bacteria translocate across the gut wall due to a lower CR. Some of these may serve as polyconal B cell activators or as antigens cross-reacting with DNA.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a disease of unknown etiology characterized by hypergammaglobulinaemia and the production of multiple autoantibodies. Amongst these anti-ds-DNA antibodies are both sensitive and specific for the disease [1]. Rises in anti-ds-DNA antibody levels were shown to parallel or precede clinical parameters of disease activity [2]. Therefore, anti-ds-DNA antibodies may play a role in the pathogenesis of exacerbations of SLE supposedly by forming immune complexes with DNA. The stimulus for the *in vivo* anti-ds-DNA antibody production in SLE, however, is still unknown.

There is increasing evidence, that in autoimmune-prone animals the composition of the indigenous intestinal microflora might play an important role in the expression of autoimmune disorders. NZB mice, that develop an autoimmune disease under conventional circumstances, do not get these autoimmune phenomena when maintained germfree directly after birth [3]. Female PVG/c rats

raised under SPF circumstances until weaning, are significantly less susceptible for the induction of autoimmune thyroiditis after thymectomy and irradiation than rats of the same strain which had been raised under conventional circumstances [4]. This indicates a role for the gut microflora in the expression of the disease. In MLR/Mp-lpr/lpr mice the first aberrant cells are found in Peyer's patches at the age of 2 weeks, later on these lymphocytes are found also in the lymph nodes, the spleen and the thymus at the age of 8 weeks [5]. Thus aberrant cells, supposed to play a role in the disease, are first found in lymph nodes draining the intestinal tract. NZB/NZW mice fed milk of cows immunized against 26 different strains of intestinal bacteria have lower titres of anti-ss-DNA antibodies; the onset of proteinuria is delayed and their life span is prolonged. Antibacterial antibodies directed against intestinal bacteria administered orally delay progression of autoimmune phenomena in these genetically susceptible mice [6]. These data from experimental models might point towards a role for intestinal antigens, perhaps of bacterial origin, in the expression of the disease. There is some evidence for a role of bacterial antigens in human SLE as well. DNA isolated from circulating immune complexes of patients with SLE hybridizes with the *Escherichia coli* lac Z gene, suggesting that DNA from circulating immune complexes is, in part, of bacterial origin [7].

Bacterial products such as lipopolysaccharides and peptidoglycans may cause polyclonal B cell activation, resulting in the production of antibodies with a repertoire similar to that of humans with systemic autoimmune disease [8]. Polyclonal B cell activation has been shown to precede the development of autoimmune phenomena in lupus-prone mice [9]. Besides autoantibody production as a result of polyclonal B cell activation, there is evidence that antigen-driven production of autoantibodies is operative in SLE [9, 10]. Antigen-driven production of anti-ds-DNA antibodies also may occur due to cross-reactivity between bacterial and human DNA [11–14]. This information suggests a role for bacteria in the pathogenesis of systemic autoimmune disease [15, 16].

Before bacteria might exert any effect on the development of autoimmune disease at all, they must gain access to their host. This access, or translocation, is hindered by the Colonization Resistance (CR) of the digestive tract. CR is the defence capacity against colonization of the digestive tract by foreign, ingested bacteria [17] and CR hinders their subsequent translocation across the intestinal wall [18, 19]. Intestinal translocation of bacteria is studied mainly in mice, rats, rabbits, dogs and pigs. However, translocation occurs in humans as well.

In humans translocation has been studied in surgery, trauma, oncology and burn patients [20–23]. In healthy humans translocation has never been shown directly. However, translocation enables contact of bacteria or bacterial fragments with the immune system. Consequently a result of this contact of intestinal bacteria with the immune system is the production of systemic antibacterial antibodies. Indeed these antibodies have been measured in healthy human individuals directed against a facultatively anaerobic *E. coli* strain that colonized their intestines after oral contamination [24]. Circulating antibodies directed against their own anaerobic faecal bacteria could also be measured in healthy human individuals, indicating that translocation of intestinal bacteria actually had happened [25].

A lower CR implies an increased chance of translocation of more species of foreign bacteria. Some of these bacteria may translocate and either serve as antigen for the production of anti-bacterial antibodies crossreacting with DNA or as polyclonal activators, and may thus form the trigger for polyclonal and antigen-specific B-cell activation in SLE. Therefore we performed a study on the quality of the CR of the intestinal microflora directed against foreign, intruding bacteria in human SLE.

SUBJECTS AND METHODS

Patients and healthy individuals

Twenty-four patients (19 women and 5 men), median age 31·5 years (range 17–66) and 19 healthy volunteers (16 women and 3 men), median age 32·0 years (range 22–58), entered this study having given written informed consent. The patients and the healthy volunteers had not received prednisone in a dosage of more than 30 mg daily in the 3 months preceding the study period nor had they had any medication influencing gastrointestinal motility or permeability. In the 1 month period before and during the sampling no one had suffered from a gastrointestinal disease or had taken antibiotics. The study was approved by the Medical Ethics Committee of the University Hospital Groningen.

Sampling

Four faecal per individual samples were collected at regular intervals over 2 weeks. The fresh faecal samples were stored at −20 °C until use.

Assessment of disease activity

Active disease was defined as the occurrence of a minor of major disease exacerbation according to previously described criteria [2] at the time of sampling. If the patient did not fulfil those criteria the disease was considered inactive. In addition, a disease activity index according to Liang and co-workers [26] was calculated at the outpatient clinic visit at the end of the 2 weeks sampling period. The mean (SD) SLE activity score according to Liang and colleagues in the patients assigned to the group with inactive SLE was 5·2 (1·76), the mean (SD) SLE activity score in patients assigned to the group with active disease was 14·4 (2·64).

Measurement of colonization resistance (CR) by comprehensive biotyping of Enterobacteriaceae

In mice the CR was originally expressed as the ^{10}log concentration of a specific potentially pathogenic bacterial species found in the faeces 2 weeks after oral contamination [27]. CR was also defined as the reciprocal value of the mean number of different biotypes of *Enterobacteriaceae* isolated from four faecal samples obtained within 2 weeks [28]. The latter method was applied here because in healthy human volunteers the two methods were shown to be significantly correlated [24]. Details of the biotyping technique have been described previously [29].

https://doi.org/10.1017/S0950268800057770 Published online by Cambridge University Press

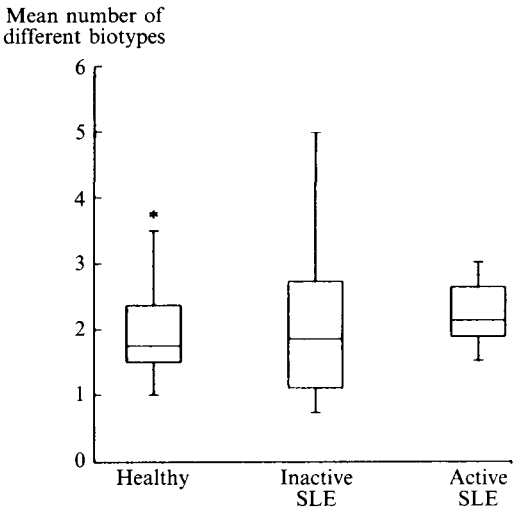

Fig. 1. Mean numbers of different biotypes of *Enterobacteriaceae* are shown for the healthy individuals, patients with inactive SLE and patients with active SLE. The inverse of the mean number of different biotypes of *Enterobacteriaceae* is indicative for the quality of the CR. The lower boundary of the box is the 25th percentile, and the upper boundary is the 75th percentile. The line in the box represents the median. The largest and smallest observed values that are not outliers are shown by lines drawn from the ends of the box to these values. Outliers are designated by an asterisk.

Each biotype number refers to a specific bacterial strain, with defined biochemical properties. Biotyping therefore reveals the composition of the aerobic (facultatively anaerobic) Gram-negative part of the faecal microflora that belongs to the *Enterobacteriaceae*.

Statistical analysis

Differences in the CR between two groups were compared with the Wilcoxon test, between three groups with the Kruskal–Wallis test, both with correction for ties.

RESULTS

The mean number of different biotypes isolated from four faecal samples in healthy individuals ($n = 19$) was 2·0, range 0·75–5. In patients with inactive SLE ($n = 16$) the mean number was 2·1, range 0·75–5 and in patients with active SLE ($n = 8$) the mean number was 2·4, range 1·5–4·25 (Fig. 1). There were no significant differences in the mean number of biotypes isolated from the faecal samples of patients and healthy individuals. The colonization resistance, however, tended to be worse in patients with active SLE than in healthy persons ($P = 0·09$, Wilcoxon one sided, with correction for ties).

The great majority of the different biotypes that were isolated from the faecal samples of both the healthy persons and the patients with active and inactive SLE consisted of strains of *E. coli*. These are common constituents of the aerobic

https://doi.org/10.1017/S0950268800057770 Published online by Cambridge University Press

(facultatively anaerobic) part of the normal faecal microflora. Klebsiella were cultured only once (0·6%) from a faecal sample of a healthy person (biotype 5255773) and twice (2·6%) from a patient with active SLE (biotypes 5215773 and 5245773). *Enterobacter cloacae* was found more often in the faecal samples of the patients than in the faecal samples of the healthy subjects. Enterobacter was found four times (5·3%) in faecal samples of patients with active SLE (biotypes 1245773, 3305573), seven times (5·2%) in faecal samples of patients with inactive SLE (biotypes 3305373, 3305573) and five times (3·3%) in faecal samples of healthy individuals (biotypes 1104572, 1305573, 3305563, 3305573).

DISCUSSION

The results of our study indicate that the CR of the intestinal microflora in SLE patients tends to be lower than in healthy individuals. This was obvious comparing patients with active SLE with healthy individuals (Fig. 1), although the differences were not significant possibly due to the rather small size of the group with active SLE. The CR is determined mainly by the indigenous microflora of the host, but also by the host him/herself. If the CR is low, more different strains of potentially pathogenic (aerobic) bacteria will colonize the digestive tract, transiently or permanently, in higher concentrations, with subsequently higher risks of translocation of the gastrointestinal wall by bacteria. In patients with active SLE the lower CR might imply that a larger diversity of bacterial antigens could invade over the gut wall than in healthy individuals which might serve as antigens for the production of anti-bacterial antibodies crossreacting with DNA or as polyclonal activators. In fact we found obvious differences between SLE patients and healthy individuals with respect to the antibody titres directed against certain indigenous gastrointestinal bacteria, supporting our hypothesis that a lower CR implies increasing translocation and antibacterial antibody production.

A role in the pathogenesis of SLE for potentially pathogenic (aerobic) bacteria has already been suggested [12]. Interestingly, crossreactivity between antibodies against klebsiella and anti-ds-DNA antibodies has been found [12]. Klebsiella were cultured only twice from faecal samples of patients with active SLE and once from a faecal sample of a healthy individual, so it is doubtful whether that species is the single bacterium that may be associated with exacerbations of SLE in our patient population. *Enterobacter cloacae* was found more often in the faecal samples of our patients than in the faecal samples of the healthy subjects. This could be due to a lower CR, since this *Enterobacter cloacae* was found only occasionally in those patients and apparently was not part of their resident intestinal microflora.

In conclusion, our data support the hypothesis that in active SLE patients the quality of the CR of the intestinal microflora is lower than in healthy individuals. A lower CR results in translocation of more species of foreign bacteria. Some of these bacteria may serve as antigen for the production of anti-bacterial antibodies crossreacting with DNA.

ACKNOWLEDGEMENTS

The study was supported by grant 89/CR/212/90 from the Dutch Foundation against Rheumatism. The hardware was provided by the Foundation 'Research of Intestinal Microflora and Decontamination'.

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