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# Healthy individuals possess circulating antibodies against their indigenous faecal microflora as well as against allogenous faecal microflora: an immunomorphometrical study

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SUMMARY

Healthy persons were shown to possess circulating antibodies of both IgA, IgG and IgM isotype directed against the bacteria of their faecal microflora, assessed by immunomorphometry. After removal, by absorption, of the fraction of antibodies directed against the autochthonous faecal bacteria or cross-reacting with allogenous faecal bacteria, there were still antibodies left directed against allogenous faecal bacteria of both the IgA, IgG and IgM isotype. However, relatively more antibodies of the IgA isotype appeared to be directed against allogenous bacteria than against indigenous faecal bacteria. Persons who reacted with specific antibodies to many bacteria of their own flora also tended to react specifically to bacteria in the allogenous microflora of the other volunteers. The patterns of antibodies directed to faecal bacteria of different morphologies (morphotypes) were unique for each individual.

## INTRODUCTION

The composition of the gut microflora is extremely complex. Acquisition of an indigenous microflora does not only depend on the type of bacteria which are ingested during and after birth, but depends also on the ability of bacteria to live in the niches of the gastrointestinal tract. This ability could be favoured by cross-reactivity between antigenic determinants of host and bacteria. In mice, for example, cross-reactivity was shown between the fetal colonic mucosa and bacteroides strains colonizing adult mice [1]. The HLA pattern of the host may also influence the composition of the microflora as was shown in a study on monozygotic and dizygotic twins with Crohn's disease [2]. The prevalence of *Klebsiella pneumoniae* in faeces was found to be partly determined by the sex and HLA state of the host [3]. Therefore, it can be expected that not only every individual has his own HLA pattern but also his own specific intestinal microflora.

In each human individual, the intestinal microflora has been found rather stable in composition. However, between individuals, the faecal flora may differ significantly on culturing [4]. Because of this inter-individual difference, an analysis of the composition of the intestinal microflora is often indicated. For example, bacteriological analysis is required in subsequent samples when the

influence of treatment with an antimicrobial drug on the endogenous flora is being studied [5, 6]. Likewise, the influence of dietary components frequently requires bacteriological analysis of the faecal flora [7]. However, since the great majority of the intestinal bacteria is strictly anaerobic, processing and culturing is very laborious. Apart from analysis by culturing, the composition of faecal flora can be studied indirectly, in several ways by determining microflora associated characteristics (MACs) [8]. These MACs involve the presence of bacterial enzymes [9] or their products [10].

In our laboratory, Meijer [11] has recently worked out a non-chemical MAC. He has developed computer software which makes it possible to determine the morphological distribution (qualitative and quantitative analysis of shapes) of faecal bacteria with great accuracy [12]. With this approach significant changes in the fraction, with more than $10^7$ bacteria/g, can be measured. Meijer's method is for example very useful for accurate monitoring of the intestinal (faecal) microflora during antibiotic treatment [13]. However, for the detection of small but essential changes in the composition of the faecal flora, such as may be caused by dietary changes, the sensitivity of morphological analysis of faecal flora needs to be increased. Therefore, we developed a method for characterization of the micromorphological composition of faecal flora in greater detail by measuring the circulating antibody response against the faecal flora by indirect immunofluorescence. For the classification of the bacteria the indirect immunofluorescence was combined with Meijer's micromorphological analysis [14]. This approach provided in addition insight into the humoral immune response to indigenous intestinal bacteria.

Bacteria composing the faecal microflora belong either to the resident indigenous microflora or to the transient allogenous or xenogenous microflora. If a person has a very stable indigenous microflora, this microflora may be regarded as part of 'self' by his immune system. Against such bacteria mainly IgM antibodies will be produced, belonging to and regulated by a network. This network is developed early in the ontogeny and consists of IgM antibodies produced by CD5-positive B cells [15]. If there is a good balance between the host and his microflora, very few if any other allogenous strains will be able to settle in the intestines of such a host for some time. Hence only rarely a specific humoral immune response with antibodies of IgA and IgG isotype will be mounted against allogenous bacteria. If a person has many antibodies of IgA and IgG isotype this could mean that his indigenous microflora is not very stable and thus could provide the chance for the immune system of mounting a specific humoral immune response against many species of allogenous bacteria, resulting in antibodies of IgA and IgG isotype during ageing against a widening range of allogenous bacterial species.

To test this hypothesis we investigated the serum antibody repertoire directed against the microflora of the digestive tract. Are such antibodies directed exclusively and specifically against their own faecal microflora or also against the faecal microflora of other unrelated individuals? Analogous to studies on HLA antigens [16], we investigated whether we could characterize human individuals with respect to the serum antibodies directed against the indigenous microflora of the intestinal tract of other volunteers after removal of antibodies in their serum directed against their own faecal bacteria by absorption.

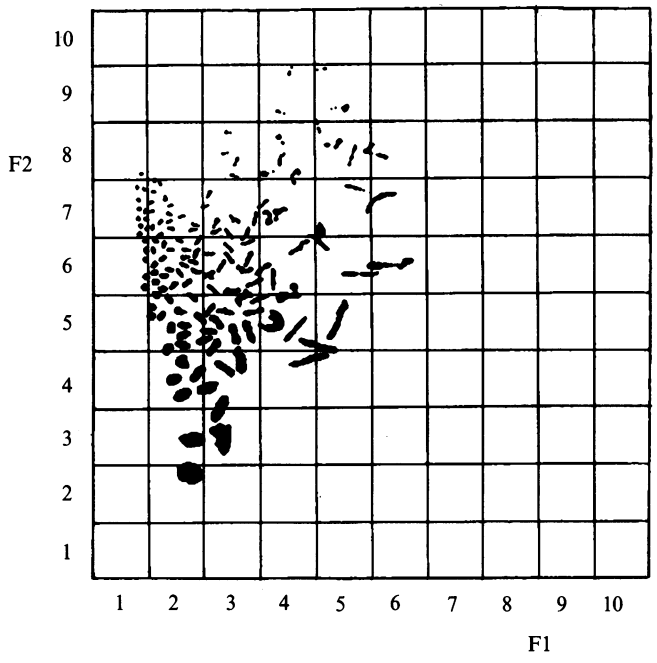


Fig. 1. Faecal objects (mainly bacteria) of a healthy volunteer are depicted according to their scores of the morphological parameters F1 and F2. The axes were divided in 10 equal parts, according to which the morphotypes were defined.

## MATERIALS AND METHODS

*Healthy individuals.* Eight healthy volunteers, 5 males and 3 females, mean age 32 years (range 22–44), participated in the study by providing 1 faecal and 1 blood sample. In the period of 1 month before the sampling, no one had suffered from a gastrointestinal disease or had taken antibiotics or any medication influencing gastrointestinal motility or permeability.

*Serum.* One hour after venepuncture blood samples were centrifuged in a Backman centrifuge type TJ-6 (Palo Alto, Calif., USA) for 10 min, at 1420 g. The serum was stored at −20 °C in aliquots of 0·2 ml.

*Faeces.* The fresh faecal samples were subdivided in aliquots of about 1 g and stored thereafter at −20 °C until use.

*Absorption.* Diluted serum (1:16, 2 × 0·4 ml) of each volunteer was absorbed three times with 0·5 ml of a faecal suspension (1:10 in demineralized water with 0·5% Tween 80 (Merck, Darmstadt, FRG), washed once with PBS) of his own faeces. Serum and faecal suspension were mixed thoroughly and incubated for 45 min at 37 °C during slow rotation. Thereafter the mixture was centrifuged in a Sigma 201 M centrifuge (Osterode, FRG) for 15 min at 11430 g. The supernatant (1 × absorbed serum 1:16) was absorbed twice more with fresh faecal suspensions in the same way. The resulting supernatant (3 × absorbed serum (1:16)) was kept at 4 °C until use the same day.

*Indirect immunofluorescence (IIF) of faecal samples.* Serum antibodies of IgA, IgG and IgM isotype were measured against the bacteria in the 'washed faeces' of the other seven volunteers by indirect immunofluorescence as described previously [14]. Instead of several serum dilutions, however, we tested only the 1:16 serum

dilution that was used in the absorption. As a positive control we assessed the presence of antibodies in the serum of each volunteer against his own microflora without previous absorption of antibacterial antibodies. We performed indirect immunofluorescence also with absorbed serum and faeces of the same volunteer as an absorption control and with faecal bacteria in PBS instead of serum as a negative PBS control.

*Immunomorphometry: reading of the immunofluorescence slides*

*Hardware.* We used a microscope (Olympus BH2, Olympus Optical Co. (Europa) GmbH, Hamburg, FRG) equipped with a phase-contrast condenser and halogen lamp (HBO 50 W, Osram, Berlin, FRG). A videocamera (Fairchild CCD 5000/1, Fairchild Weston Systems Inc., Sunnyvale, Calif., USA) was placed on top of the microscope. The camera was connected to an 80386-based AT compatible computer with a Matrox MVP-AT(/NP) image processor board and a monitor. Images recorded by the videocamera were displayed on a high-resolution colour screen.

*Software.* The image acquisition software was developed specifically for this application in our laboratory. The analysis package used was an adaptation of the morphological package developed by Meijer and co-workers. [11]. For each combination of faeces of a volunteer and indigenous-flora-absorbed serum of another volunteer, between 500 and 1000 bacteria were analysed individually both with regard to their individual morphology and to the occurrences of fluorescence due to positive indirect immunofluorescence. The bacteria analysed belonged mainly to the anaerobic indigenous microflora of the respective volunteers.

Based on micromorphological properties the bacteria were grouped in 'morphotypes' (Fig. 1). A 'morphotype' can be defined as the cluster of morphologically different related objects that is enclosed by each square of a raster placed over the entire population of objects (bacteria) in a faecal preparation. The first number encoding each morphotype refers to Factor 1 ($x$-axis), the second number to Factor 2 ($y$-axis), and the third number to Factor 3 ($z$-axis) not shown in Fig. 1. Factor 1 and 2 are each divided in 10 equal parts, Factor 3 in 2 equal parts. The codes of the morphotypes with the representative forms of the objects belonging to them are depicted in Fig. 2.

Within each of these morphotypes the median of fluorescence intensity of the objects (bacteria) was measured. The median was calculated only if the morphotype contained a sufficient number of objects. A minimum of ten objects was required. We calculated the median error of the medians of fluorescence in a total of 140 morphotypes and added twice this value to the PBS control fluorescence as a threshold for immunofluorescence in that morphotype. Fluorescence was considered positive if the median fluorescence in a certain morphotype exceeded the threshold. In this way it was possible to assess humoral immune reactivity of volunteers against the faecal microflora of others.

*Statistical analysis.* We compared the systemic humoral reactivity of the IgA, IgG and IgM isotype against their own faecal bacteria versus allogenous faecal bacteria by performing the Spearman rank correlation test. The volunteers were ranked according to the number of times antibodies were directed against different faecal morphotypes of their own or of the other volunteers.

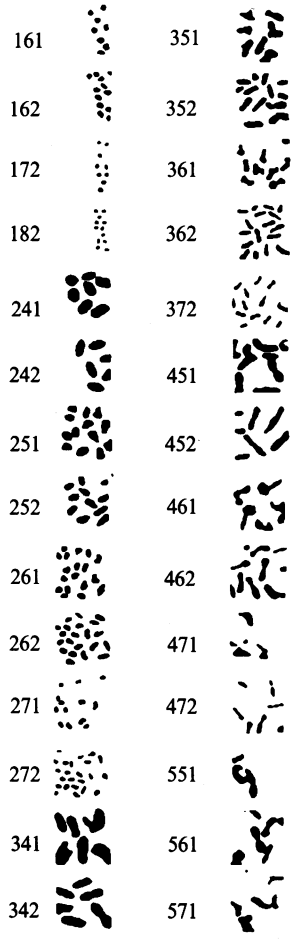


Fig. 2. Representative forms of objects belonging to the 28 morphotypes.

## RESULTS

Antibodies of the IgA, IgG and IgM isotype were directed against bacteria of the autochthonous faecal flora, as was shown for IgG in Fig. 3. Also after absorption of the serum with the autochthonous faecal microflora antibodies directed against some faecal bacteria of unrelated individuals were found. The number of morphotypes with positive immunofluorescence for the isotypes IgA, IgG and IgM is shown in Table 1. The absorption procedure, which was performed three times for each serum sample, was not always completely successful. It appeared that after three absorptions positive immunofluorescence could be measured against 10% of the morphotypes (Tables 2A–C).

Taking into account the data of all absorbed sera versus faecal samples of the other volunteers (Table 1), we found 117 times positive immunofluorescence against morphotypes for the IgA isotype, 117 times for the IgG isotype and 82 times for the IgM isotype. The ratio of the number of times of positive immunofluorescence against faecal morphotypes for IgA, IgG and IgM is 1 : 1 : 0·70. The number of times positive immunofluorescence was measured against the morphotypes for the isotypes IgA, IgG and IgM was shown in Table 3. For the

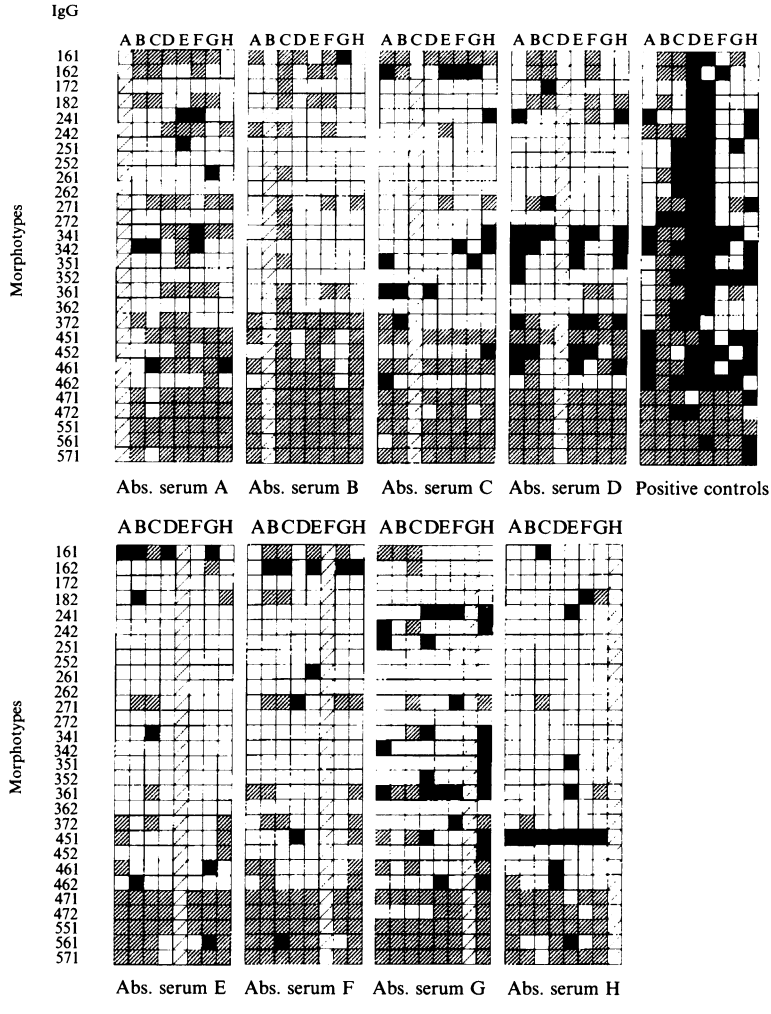


Fig. 3. The serum IgG antibody responses of volunteers A–H against the morphotypes of the faecal microflora of the volunteers A–H are shown, after absorption of the serum of each volunteer with his/her own faecal microflora. As a positive control the serum IgG antibody responses of the volunteers A–H against his/her own microflora are depicted in the upper right block. The boxes are hatched if a morphotype contained less than 10 bacteria. The boxes are black in case of positive immunofluorescence and white in case of negative immunofluorescence.

positive controls (unabsorbed sera) we found 37 times positive immunofluorescence for the IgA isotype, 87 times for the IgG isotype and 78 times for the IgM isotype. The ratio of the number of times of positive immunofluorescence against faecal morphotypes for IgA, IgG and IgM is 0·43 : 1 : 0·90. The number of times positive immunofluorescence was measured against the morphotypes for the isotypes IgA, IgG and IgM was shown in Table 4.

We found a positive correlation between the number of times positive humoral immune responses were mounted against faecal morphotypes of the autochthonous flora versus allogenous flora for the IgA isotype ($P < 0{\cdot}05$), but not for the IgG and the IgM isotypes.

Table 1. *Number of morphotypes with positive immunofluorescence of the IgA, IgG and IgM isotype respectively for each combination of absorbed serum of the healthy individuals A–H, displayed horizontally, and faecal samples of the healthy individuals A–H, displayed vertically*

| | Absorbed serum of volunteer | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Faeces | A | B | C | D | E | F | G | H | Σ |
| IgA | | | | | | | | | |
| A | — | 0 | 1 | 13 | 0 | 2 | 2 | 6 | 24 |
| B | 3 | — | 1 | 4 | 0 | 0 | 1 | 1 | 10 |
| C | 0 | 1 | — | 0 | 0 | 0 | 0 | 1 | 2 |
| D | 1 | 1 | 2 | — | 0 | 1 | 2 | 3 | 10 |
| E | 5 | 0 | 2 | 5 | — | 0 | 5 | 0 | 17 |
| F | 8 | 0 | 2 | 3 | 0 | — | 5 | 0 | 18 |
| G | 6 | 0 | 3 | 1 | 0 | 0 | — | 1 | 11 |
| H | 1 | 2 | 4 | 11 | 1 | 1 | 5 | — | 25 |
| Σ | 24 | 4 | 15 | 37 | 1 | 4 | 20 | 12 | 117 |
| IgG | | | | | | | | | |
| A | — | 0 | 4 | 8 | 1 | 0 | 4 | 1 | 18 |
| B | 1 | — | 2 | 3 | 3 | 1 | 0 | 1 | 11 |
| C | 2 | 0 | — | 3 | 1 | 2 | 0 | 2 | 10 |
| D | 0 | 0 | 0 | — | 1 | 2 | 6 | 3 | 12 |
| E | 2 | 0 | 1 | 6 | — | 2 | 3 | 5 | 19 |
| F | 3 | 0 | 2 | 3 | 0 | — | 4 | 2 | 14 |
| G | 1 | 1 | 2 | 0 | 3 | 1 | — | 2 | 10 |
| H | 1 | 0 | 4 | 7 | 0 | 1 | 10 | — | 23 |
| Σ | 10 | 1 | 15 | 30 | 9 | 9 | 27 | 16 | 117 |
| IgM | | | | | | | | | |
| A | — | 1 | 0 | 2 | 0 | 1 | 0 | 0 | 4 |
| B | 0 | — | 2 | 1 | 3 | 3 | 0 | 3 | 12 |
| C | 0 | 1 | — | 0 | 0 | 0 | 3 | 2 | 6 |
| D | 3 | 1 | 0 | — | 2 | 1 | 0 | 8 | 15 |
| E | 4 | 0 | 1 | 0 | — | 2 | 1 | 2 | 10 |
| F | 3 | 1 | 3 | 0 | 2 | — | 3 | 2 | 14 |
| G | 1 | 3 | 0 | 1 | 2 | 2 | — | 1 | 10 |
| H | 1 | 1 | 2 | 1 | 2 | 0 | 4 | — | 11 |
| Σ | 12 | 8 | 8 | 5 | 11 | 9 | 11 | 18 | 82 |

The pattern of antibodies against faecal bacterial morphotypes of other unrelated individuals was different for each individual as was illustrated for IgG in Fig. 3. Volunteer C for example, reacted predominantly to faecal morphotypes of volunteers A and H, whereas hardly any reactivity was found against the faecal morphotypes of volunteers D and E. Although volunteer C had no antibodies against his own morphotype 452, he reacted positively against bacteria of morphotype 452 of volunteer H. Volunteer F did not have IgG antibodies left after absorption that were bound by the faecal morphotypes of volunteer A. The degree of the humoral immunoreactivity of volunteers towards faecal morphotypes of other volunteers can be read from Fig. 4. Some sera of some volunteers appeared to have very few antibodies against allogenous faecal morphotypes of other volunteers left after absorption with their own faecal microflora. For IgA, for

Table 2A–C. *Faecal morphotypes of each individual (A–H) against which still positive immunofluorescence (+) of the IgA, IgG and IgM isotype respectively was measured after three absorptions of serum of each individual with the indigenous faecal microflora*

| Morphotype IgA | Volunteers A | B | C | D | E | F | G | H | Σ |
|---|---|---|---|---|---|---|---|---|---|
| 161 | | | | | | | | | 0 |
| 162 | | | | | | | | | 0 |
| 172 | + | | | | | | | | 1 |
| 182 | | | | | | | | | 0 |
| 241 | | | | | | | | | 0 |
| 242 | | | | | | | + | | 1 |
| 251 | | | | + | | | + | | 2 |
| 252 | | | | | | | | | 0 |
| 261 | | | | | | | | | 0 |
| 262 | | | | | | | | | 0 |
| 271 | | | | | | | | | 0 |
| 272 | | | | | | | | | 0 |
| 341 | | | | + | | | | | 1 |
| 342 | | | | | | | | | 0 |
| 351 | | | | | | | + | | 1 |
| 352 | | | | | | | + | | 1 |
| 361 | + | | | + | | + | | + | 4 |
| 362 | | | | | | | | | 0 |
| 372 | | | | | | | | | 0 |
| 451 | | | | | | | | | 0 |
| 452 | | | | + | | | | | 1 |
| 461 | | | | + | | | | | 1 |
| 462 | + | | | | | | | | 1 |
| 471 | | | | | + | | | | 1 |
| 472 | | | | | | | | | 0 |
| 551 | | | | | | | | | 0 |
| 561 | | | + | | | | | | 1 |
| 571 | | | | | | | | | 0 |

| Morphotype IgG | Volunteers A | B | C | D | E | F | G | H | Σ |
|---|---|---|---|---|---|---|---|---|---|
| 161 | | | | | + | | + | | 2 |
| 162 | | | | | | | | | 0 |
| 172 | | | | + | | | | | 1 |
| 182 | | | | | + | | + | | 2 |
| 241 | | | | + | | | | | 1 |
| 242 | | | | | | | | | 0 |
| 251 | | | | | | | + | | 1 |
| 252 | | | | | | | | | 0 |
| 261 | | | | | | | | | 0 |
| 262 | | | | | | | | | 0 |
| 271 | | | | | | | | | 0 |
| 272 | | | | | | | | | 0 |
| 341 | + | | | + | | | + | | 3 |
| 342 | | | | | | | | | 0 |
| 351 | | | | + | | | + | | 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 352 | | | | | | | + | | 1 |
| 361 | | | | | | | | | 0 |
| 362 | | | | | | | | | 0 |
| 372 | | | | | | | | | 0 |
| 451 | + | | | | + | | + | + | 4 |
| 452 | | | | | | | | | 0 |
| 461 | + | | | | | | | | 1 |
| 462 | | | | | + | | | | 1 |
| 471 | | | | | | | + | | 1 |
| 472 | | | | | | | | | 0 |
| 551 | | | | | | | | | 0 |
| 561 | | | | | + | | | + | 2 |
| 571 | | | | | | | | + | 1 |

| | Volunteers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Morphotype IgM | A | B | C | D | E | F | G | H | Σ |
| 161 | | | | | | | | | 0 |
| 162 | | | + | | | | | | 1 |
| 172 | | | | | | | | | 0 |
| 182 | | | | | | | | | 0 |
| 241 | | | | | | | | | 0 |
| 242 | + | | | | + | | | | 2 |
| 251 | | | | | | | | | 0 |
| 252 | | | | | | | | | 0 |
| 261 | | | | | | | | | 0 |
| 262 | | | | | | | | | 0 |
| 271 | | | | | | | + | | 1 |
| 272 | | + | | | | | | | 1 |
| 341 | + | | | | | | | | 1 |
| 342 | + | | | | | | | | 1 |
| 351 | | | | | | | | | 0 |
| 352 | | | | | | | | | 0 |
| 361 | | | | | | | + | | 1 |
| 362 | | | | | | | | | 0 |
| 372 | | | | | | | | | 0 |
| 451 | + | | | + | + | | | | 3 |
| 452 | | | | | | | | | 0 |
| 461 | + | | + | | | | + | | 3 |
| 462 | | | | | | | | | 0 |
| 471 | | | | | | | | | 0 |
| 472 | | | | | | | | | 0 |
| 551 | | | | | | | | | 0 |
| 561 | | | | | | | | | 0 |
| 571 | | | | | | | | | 0 |

example, it shows that volunteer D is most reactive towards allogenous faecal bacterial morphotypes and volunteers B, E and F are least reactive. Faecal flora contain bacteria immunogenic for other volunteers. The degree of immunogenicity of each faecal flora is shown in Fig. 5.

## DISCUSSION

Each individual volunteer appeared to have antibodies directed against the bacteria of his own faecal microflora. These antibodies were of the IgM class, as well as of the IgG and IgA class. This might mean that the humoral response

Table 3. *Number of times positive immunofluorescence was against allogenous faecal microflora and against the respective morphotypes (161–571) after three absorptions of each serum with its corresponding indigenous faecal microflora for the isotypes IgA, IgG and IgM*

| Morphotype | IgA | IgG | IgM | Morphotype | IgA | IgG | IgM |
|---|---|---|---|---|---|---|---|
| 161 | 7 | 6 | 0 | 351 | 12 | 6 | 4 |
| 162 | 7 | 9 | 2 | 352 | 10 | 3 | 0 |
| 172 | 1 | 1 | 0 | 361 | 15 | 8 | 4 |
| 182 | 4 | 2 | 4 | 362 | 2 | 0 | 0 |
| 241 | 7 | 10 | 5 | 372 | 1 | 6 | 3 |
| 242 | 4 | 2 | 5 | 451 | 0 | 11 | 9 |
| 251 | 1 | 3 | 0 | 452 | 5 | 7 | 5 |
| 252 | 4 | 0 | 0 | 461 | 6 | 7 | 12 |
| 261 | 0 | 2 | 1 | 462 | 6 | 4 | 5 |
| 262 | 0 | 0 | 0 | 471 | 1 | 0 | 1 |
| 271 | 3 | 3 | 6 | 472 | 0 | 0 | 0 |
| 272 | 0 | 0 | 3 | 551 | 0 | 0 | 0 |
| 341 | 11 | 11 | 6 | 561 | 1 | 3 | 2 |
| 342 | 8 | 11 | 2 | 571 | 0 | 0 | 3 |

Table 4. *Number of times positive immunofluorescence of each serum against its corresponding indigenous faecal morphotypes (161–571) was found for the isotypes IgA, IgG and IgM*

| Morphotype | IgA | IgG | IgM | Morphotype | IgA | IgG | IgM |
|---|---|---|---|---|---|---|---|
| 161 | 0 | 2 | 0 | 351 | 3 | 3 | 5 |
| 162 | 1 | 2 | 2 | 352 | 4 | 6 | 6 |
| 172 | 1 | 2 | 3 | 361 | 2 | 2 | 2 |
| 182 | 2 | 2 | 2 | 362 | 2 | 3 | 4 |
| 241 | 3 | 4 | 4 | 372 | 1 | 2 | 2 |
| 242 | 0 | 3 | 2 | 451 | 2 | 4 | 1 |
| 251 | 2 | 4 | 4 | 452 | 3 | 5 | 5 |
| 252 | 2 | 3 | 3 | 461 | 0 | 5 | 2 |
| 261 | 0 | 3 | 2 | 462 | 2 | 6 | 5 |
| 262 | 0 | 3 | 4 | 471 | 0 | 1 | 0 |
| 271 | 1 | 3 | 4 | 472 | 1 | 2 | 2 |
| 272 | 1 | 4 | 3 | 551 | 0 | 0 | 0 |
| 341 | 2 | 5 | 4 | 561 | 0 | 2 | 2 |
| 342 | 2 | 6 | 6 | 571 | 0 | 1 | 0 |

against bacteria of one's own microflora did not consist solely of predominantly polyspecific antibodies (IgM) but also of specific antibodies (IgA, IgG). After removal by absorption of antibodies directed against the autochthonous bacteria and perhaps partly cross-reacting with the faecal bacteria of other persons, almost every individual appeared to have antibodies against allogenous faecal bacteria left. However, the absorption was not always complete. Although aspecific binding with low avidity cannot be excluded, the most likely explanation for the occasional incomplete absorption is, that the antibody titre in the sera of these volunteers was high in relation to the amount of antigen (objects in the morphotypes) in the faecal preparations.

The antibodies against allogenous bacteria were again of the IgM, IgG and IgA isotype. The ratios, however, of positive immunofluorescence against the faecal

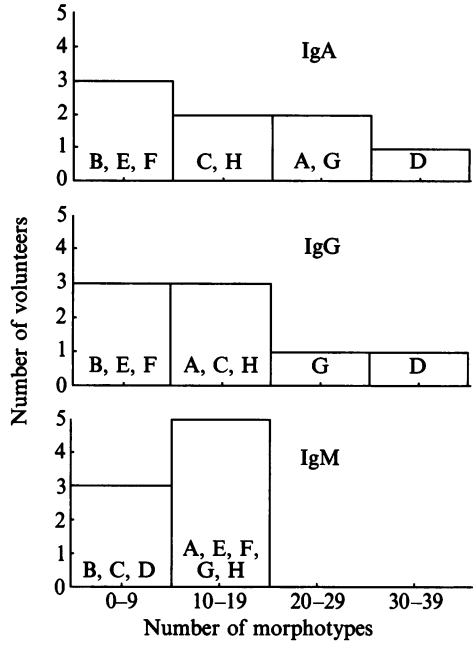


Fig. 4. After absorption of his serum with autochthonous faeces each volunteer (A–H) has serum antibodies left against allogenous faecal morphotypes. The number of allogenous faecal morphotypes to which serum antibodies are directed by each volunteer (A–H) is shown, thereby illustrating the degree of humoral immunoreactivity of each volunteer towards allogenous faecal morphotypes.

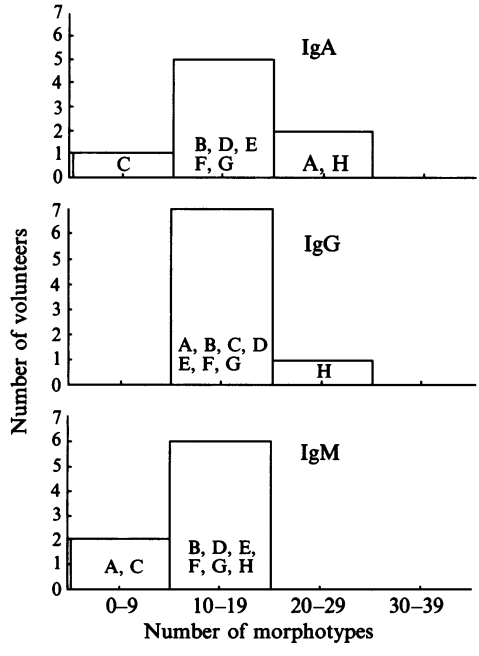


Fig. 5. Some faecal flora contain bacteria immunogenic to other volunteers. The degree of immunogenicity of each faecal flora is illustrated by showing the number of faecal morphotypes to which the other volunteers appeared to possess serum antibodies.

morphotypes were different for antibodies directed against the autochthonous faecal microflora (IgA:IgG:IgM = 0·43:1:0·90). The isotypes of antibodies left after absorption with the autochthonous bacteria and directed against allogenous faecal microflora appeared in a ratio of IgA:IgG:IgM = 1:1:0·70. This could mean that a positive serum IgA response towards allogenous faecal bacteria is more frequent than that towards the autochthonous faecal bacteria. More often allogenous faecal bacteria produce a specific antibody response than one's own faecal bacteria. This confirms early findings of Foo and Lee in several species of animals [17]. Some of these allogenous faecal bacteria might temporarily have been part of the microflora and have caused a humoral immune response during this colonization period. This response of the host may have occurred because these bacteria did not belong to the indigenous flora.

Each individual displayed another pattern of antibodies against faecal bacterial morphotypes of other unrelated individuals as well as against his own faecal bacterial morphotypes. If the composition of the faecal microflora depends, amongst others, upon cross-reactivity either in the HLA type epitopes of the host or host tissue antigens, interindividual differences in antibody patterns are to be expected in a heterogeneous group of human subjects.

The positive correlation between the number of times positive IgA responses were mounted against the faecal morphotypes of one's own flora versus the allogenous flora supports our hypothesis. If a person has many antibodies of IgA and IgG isotype this could mean that his indigenous microflora is not very stable and thus could provide the chance for the immune system to mount a specific humoral immune response against many species of allogenous bacteria. This might result in antibodies of IgA and IgG isotype during ageing against a widening range of allogenous bacterial species. Persons with a large allogenous component in their own microflora to which specific humoral responses are elicited also react specifically to many other allogenous bacteria. For IgG for example a volunteer such as B had a faecal microflora of intermediate immunogenicity (Fig. 5) against which he had no antibodies. Still volunteer B had a few antibodies against faecal microflora of others. Volunteer D had many antibodies against his own microflora as well as against the microflora of others (Fig. 3). In fact we saw this effect for IgG antibodies in 6/8 persons; therefore, the results for IgG were not significant. Volunteer E, for example, was quite exceptional in having many antibodies against his own microflora and hardly any antibodies against the microflora of others.

The humoral reactivity of the host against his gastrointestinal microflora might play a role in certain circumstances leading to disease. Determination of pathogenic faecal serotypes might be helpful in diagnosis and therapy of such diseases, once proof has been obtained that such serotypes actually play a role in the pathogenesis of the disease. The fact, however, that volunteer C, for example, had no antibodies against his own morphotype 452, but reacted positively against morphotype 452 of volunteer H indicates that the classification in morphotypes does not parallel a possible classification in serotypes. Therefore, our method cannot be used for the establishment of a system for identification of antibodies directed against serotypes of morphologically defined groups of faecal bacteria.

To conclude, with immunomorphometry circulating antibodies were detected against the indigenous faecal bacteria as well as against allogenous faecal bacteria.

Some of these antibodies are of the polyspecific (IgM) type and some are specific (IgA, IgG). Relatively more antibodies of the IgA isotype appeared to be directed against allogenous faecal bacteria in comparison with the indigenous faecal bacteria. Persons who reacted frequently with specific antibodies to their autochthonous flora, also tended to react specifically to the allogenous microflora of the other volunteers. The pattern of antibodies directed against faecal morphotypes was unique for each individual.

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