or endocrine disease. Such patients are thought to lack one or more of the factors implicated in the full expression of gastric atrophy or endocrine disease.

Our findings are consistent with several reports of associations between HLA antigens and pernicious anaemia.⁶⁻¹² We attribute the discrepancies between other reports to the differing proportions of patients with the three HLA patterns.

The relative risks for HLA-B-locus antigens in pernicious anaemia were small. This suggests that there are other important determinants of the condition, possibly including non-HLA-linked genes. Our observations suggest that susceptibility to pernicious anaemia might be partly determined by genes in linkage disequilibrium with the HLA-B-locus,¹⁷ but they might also indicate an effect of HLA-linked genes on the length of survival in patients with pernicious anaemia. The excess of heterozygotes for certain HLA-B-locus antigens suggests that these genes show overdominance or additive effects similar to those in diabetes mellitus.¹⁶

The contrasting HLA patterns in clinically defined subgroups of patients with pernicious anaemia might indicate heterogeneity in the pathogenesis of pernicious anaemia, and we suggest that pernicious anaemia is determined by the action of autoimmune and other factors on a susceptible gastric mucosa.¹⁸

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Partial antibiotic decontamination

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Summary

Partial antibiotic decontamination and reverse isolation were carried out in nine patients undergoing bone marrow transplantation. The aim of this approach was to eradicate the patient's endogenous potentially pathogenic bacteria while preserving the anaerobic flora of the gut, which help to prevent recolonisation. No exogenous infections developed, and only one patient developed an infection associated with endogenous recolonisation. Colonisation resistance seemed normal in patients during partial antibiotic decontamination. This form of decontamination deserves further study in patients with immunodepression.

Introduction

The micro-organisms that most often cause infections in patients with impaired resistance are *Escherichia coli*, *Klebsiella*

spp, and other Enterobacteriaceae, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*.¹ Attempts to prevent infection by total antibiotic decontamination eliminate not only these potential pathogens but also the normal anaerobic gut flora. Animal studies have shown that these anaerobic bacteria strongly inhibit exogenous or endogenous bacterial colonisation.^{2,3} This bacteriostatic ability, or colonisation resistance, is severely reduced in decontaminated patients who have been deprived of their anaerobic flora. If reverse isolation or total antibiotic decontamination is not completely successful in these patients micro-organisms can easily colonise or recolonise the gut in abnormally high numbers, sometimes causing diarrhoea or infection.^{4,5}

Other effects of total antibiotic decontamination are nausea, changed gut motility, atrophy of the villi, and malabsorption. Moreover, the colon tends to become distended, and diarrhoea-like stools are formed.^{6,7} To overcome some of these problems in nine patients undergoing bone marrow transplantation we tried to eradicate most of the common aerobic potential pathogens while preserving the anaerobic flora of the gut (partial decontamination), since we considered that these patients had no special predisposition to anaerobic infection.⁸

Patients and methods

The three women and six men (mean age 21 (range 16-42) years) were suffering from acute myeloid leukaemia, acute lymphatic leukaemia, or severe aplastic anaemia. They were all treated with immunosuppressive and cytostatic drugs before undergoing bone marrow transplantation.

Most of the patients had had severe granulocytopenia for several weeks ($<0.5 \times 10^6/l$ for an average of 45 days; $<0.1 \times 10^6/l$ for an

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average of 21 days), and all were considered to be very prone to bacterial and fungal infections. Three patients (cases 4, 7, and 8) had had infections on admission. In their first week in hospital two (cases 4 and 8) were treated with intravenous cloxacillin for 10 days to cure dormant sinusitis and purulent tonsillitis (*Staph aureus*), and the third patient (case 7) was treated with intravenous co-trimoxazole and a combination of intravenous ampicillin and cloxacillin for septicaemia (*E coli*) and sinusitis (*Staph aureus*). Over several weeks the clinical condition of most of the patients deteriorated as a result of the cytostatic treatment, total body irradiation, surgical treatment, and graft-versus-host disease. During these episodes the partial decontamination regimen had to be stopped for some days, because the patients could not take the drugs by mouth.

Microbiological methods—A bacteriological inventory of the patient's endogenous microflora was carried out twice a week during partial antibiotic decontamination. Samples of faeces and urine and swabs from the nose, oropharynx, ear, axilla, groin, and prepuce or vagina were cultured. Suspect areas such as wounds, tonsils, or inflamed tissue were also swabbed. The material was cultured on selective and non-selective media (Oxoid, London; and Difco, Detroit). Faeces were also cultured quantitatively, by serial dilution in brain-heart infusion broth (Oxoid), and qualitatively, by subculturing these dilutions on various selective and non-selective media (Oxoid and Difco) after overnight incubation. Bacteria, yeast, and fungal species were identified by routine bacteriological methods whenever possible. Enterobacteriaceae were further typed on the basis of the biochemical activity pattern (API system, La Balme, France).⁹ An inventory of the faecal anaerobes was carried out three or four times during the decontamination and isolation period. The anaerobes were cultured according to the methods of Wensinck *et al*¹⁰ and Davis *et al*.¹¹ After the anaerobes had been isolated we typed them by measuring several biochemical and morphological indices (volatile fatty acid production, fermentative reactions, ammonia, indole and gas production, catalase activity).¹² In most cases we could not identify the actual species.

Decontamination—The naso-oro-pharyngeal region was sprayed with an aqueous solution of 3% gentamicin four times a day. Amphoteribin B 3%, neomycin 3%, and cephaloridine 3%, contained in Orabase, were applied to the mouth; 1 g of this ointment was applied four times a day. To decontaminate the gastrointestinal tract 250 mg neomycin or kanamycin, 100 mg polymyxin B or colistin, 1000 mg nalidixic acid, and 250-500 mg amphotericin or miconazole were given by mouth four times a day. The skin was washed daily with Povidone-iodine soap and water containing 0.5% chlorhexidine. The prepuce or vulval and vaginal region was treated with a cetyl-base cream containing the same combination of antibiotics as the one used for the mouth. Macroscopically detectable *C albicans* that persisted in the mouth was treated with a gentian violet solution. Persisting potential pathogens on the skin were treated daily with silver sulphadiazine.

Isolation—The patients were nursed in strict reverse isolation (laminar down-flow isolator or a comparable system¹³), except for three patients (cases 1, 5, and 7) who were nursed for several weeks under semi-isolation—that is, they were allowed to leave their isolators protected only by a well-fitting mask. Two of these patients (cases 5 and 7) were allowed to go outdoors without masks. All patients were fed sterile food prepared in a special kitchen. The food was checked regularly for bacterial contamination in our bacteriological laboratory.

Results

CASE 5

The figure shows the bacteriological status of this patient with aplastic anaemia during partial antibiotic decontamination. Shortly after admission his spleen was removed to reduce the need for transfusion. He was strictly isolated and partially decontaminated. The potential pathogens were rapidly eradicated and his anaerobic flora seemed to be normal during decontamination. This could be seen in Gram-stained direct faecal smears. At the end of the first period (period 1) *Bacteroides* spp (10^{10} /g faeces), *Eubacterium* spp (10^{10} /g faeces), four species of *Clostridium* (10^{11} , 10^{10} , 10^6 , and 10^4 /g faeces), and two species of anaerobic cocci (10^4 and 10^{11} /g faeces) were isolated from the faeces.

The patient had no nausea or diarrhoea due to decontamination. His stools had a normal consistency, and his appetite was normal, though he sometimes refused to swallow the antibiotics. *Streptococcus*

spp persisted in the mouth during decontamination. At the end of period 1 *C albicans* was isolated for the first time. After splenectomy the peripheral-blood picture improved (granulocytes $>0.5 \times 10^6$ /l; platelets $>10 \times 10^6$ /l), and he was therefore discharged and followed up as an outpatient, without isolation or decontamination treatment (period 2). During period 2 he was colonised by *P mirabilis* and *E coli* type 2, but not by *E coli* type 1 or *Kl pneumoniae*, which had been eradicated by partial decontamination in period 1. About a month later the numbers of granulocytes and thrombocytes fell to $<0.2 \times 10^6$ /l and $<10 \times 10^6$ /l respectively, and he was readmitted for bone-marrow transplantation (period 3).

Case 5. Presence and elimination of aerobic micro-organisms during periods of partial decontamination. Horizontal bars indicate continuing presence of bacterial species. Dots indicate bacterial species present in low concentration in some cultures.

Again partial decontamination succeeded in eradicating the aerobic potential pathogens with the exception of two types of streptococci and *C albicans* in the mouth. Two weeks after the transplantation the patient developed severe diarrhoea due to graft-versus-host disease. The levels of the anaerobic bacteria fell to extremely low values. Gram-stained faecal smears and quantitative anaerobic cultures showed that the anaerobic flora spontaneously recovered when the stools became normal again, despite continued partial antibiotic decontamination. The anaerobic species were the same as those found in period 1. In period 4 the isolation was reduced because of psychological problems. During this period no exogenous colonisation could be detected.

Five months after bone-marrow transplantation the patient was discharged. His peripheral blood picture was normal but there was still considerable immune deficiency due to abnormally low serum immune globulin levels. The species of bacteria eliminated during his second period in hospital (periods 3 and 4) did not recolonise after the end of decontamination.

OVERALL RESULTS

In the other patients, with the exception of *E coli* and *Klebsiella* spp in case 6, most of the potential pathogens were eliminated (table 1). In four patients *Str faecalis* persisted. *C albicans* and *Streptococcus* spp persisted in the mouths of most patients but were absent or occurred only in very low concentrations in the faeces ($<10^3$ /g faeces), except during periods of diarrhoea—that is, when the anaerobic flora were reduced the numbers increased (10^4 - 10^8 /g faeces).

Staph albus persisted on the skin of all patients. The anaerobic gut flora of all patients included several anaerobic species that were resistant to partial decontamination. This was seen in direct faecal smears and confirmed by anaerobic cultures. *Bacteroides* spp (10^8 - 10^{12} /g faeces), *Eubacterium* spp (10^{10} - 10^{11} /g faeces), *Clostridium* spp (10^4 - 10^{11} /g faeces), *Bifidobacteria* (10^2 - 10^8 /g faeces), and anaerobic cocci (10^4 - 10^{11} /g faeces) were isolated from the faeces.

TABLE 1—Eliminated and persisting bacteria in patients who underwent partial decontamination

Case No:	1	2	3	4	5*	5†	6	7	8	9
<i>E coli</i>	E	E	E	E	E	E	P	E	E	E
<i>Klebsiella</i> spp.	E	E	E	E	E	E	P	E	E	E
Other Enterobacteriaceae	E	E								
<i>Ps aeruginosa</i>										
<i>Staph aureus</i>		E	E	E	E	E	E	E	E	E
<i>Str faecalis</i>	P	P	E	E	E	E	P	P	P	P
<i>C albicans</i>										
<i>Dip pneumoniae</i>	E	E	E	E	E	E	E	E	E	E
<i>Neisseria</i> spp.	E	E	E	E	E	E	P	P	P	P
<i>Streptococcus</i> spp.	P	P	P	P	P	P	P	P	P	P
<i>Staph epidermidis</i>	P	P	P	P	P	P	P	P	P	P

*Results in period 1 (see figure). † Results in periods 3 and 4 (see figure).
E = eliminated; P = persisting. Blanks indicate that the organism was absent.

TABLE II—Infections seen during partial antibiotic decontamination

Case No	Disease*	Duration of partial decontamination (days)	Bacterial or fungal infections during decontamination†	Course during period of study
1	ALL	80		Relapse; died on day 205
2	AA	95		Alive (day 350)
3	AA	110		Alive (day 300)
4	AML	115		Cytomegalovirus: interstitial pneumonia; died on day 115
5	AA	50 + 160	Septicaemia days 175-180	Alive (day 285)
6	AA	235		Alive (day 325)
7	AA	88		Alive (day 150)
8	AML	60		Graft-versus-host disease; died on day 60
9	AA	30		Alive (day 30)

†ALL = acute lymphatic leukaemia; AML = acute myeloid leukaemia; AA = aplastic anaemia.

†Infections during first week of admission not included.

Table II shows the infections seen during partial decontamination. In case 6 septicaemia (*Kl pneumoniae*) was diagnosed on day 175. During this period of infection the patient had no granulocytes. Blood cultures became negative after a week of treatment with intravenous co-trimoxazole and granulocyte transfusions.

Discussion

Partial antibiotic decontamination rapidly eliminated the common potential pathogens in these patients undergoing bone-marrow transplantation. This proved to be a lasting eradication of bacteria because none of the discharged patients suffered endogenous recolonisation by the eliminated species of micro-organisms. None of the patients complained of nausea due to decontamination. Their appetites were normal and stools had a normal consistency. The anaerobic flora of the gut seemed to remain normal during partial decontamination.

Candida often persisted in the mouth, but no patient suffered inflammation of the gums or a generalised candidiasis. We did not consider that persisting *Staph albus* on the skin and *Streptococcus* spp in the mouth were pathogenic in these patients, and so we made no special effort to eradicate them.

Candida spp and *Streptococcus* spp occurred sporadically in the faeces, but these micro-organisms were probably unable to colonise the gut in the presence of the persisting anaerobic flora. During periods of diarrhoea due to graft-versus-host disease four patients (cases 1, 4, 5, and 6) showed a dramatic reduction in their anaerobic flora coupled with increasing amounts of streptococci and *Candida* spp in the faeces. When the stools returned to normal the anaerobic flora recolonised the gut spontaneously despite decontamination, and the normal levels of *Candida* and streptococci were restored. This phenomenon was probably a manifestation of colonisation resistance. Partial decontamination failed seriously in case 6. *Kl pneumonia* and *E coli*, though sensitive to treatment in vitro, persisted

on his skin (groins) but were not found in the faeces over 200 days, despite septicaemia caused by *Klebsiella* from day 175 to day 185. On day 225 he developed severe diarrhoea. Biopsy samples from the mucosal wall of the colon showed signs of graft-versus-host disease. About a week later *E coli* recolonised the gut endogenously, probably because of the patient's refusal to take the antibiotics and his reduced colonisation resistance due to the reduced level of the anaerobic flora usually seen during a period of diarrhoea.

Although it is intended to eradicate all bacteria and fungi, total antibiotic decontamination usually fails to do so. Partial antibiotic decontamination, which is intended to eradicate only potential pathogens, offers the advantage that colonisation resistance is preserved. This means that if decontamination or isolation fails there is less risk of exogenous or endogenous recolonisation and no risk that decontamination will result in a totally disturbed endogenous flora accompanied by diarrhoea and infection. Successful partial decontamination requires a combination of poorly absorbed antimicrobial drugs, however, because the use of a single drug—for example, nalidixic acid—for selective elimination reduces only the enterobacteriaceae species, and does not affect enterococci, *Pseudomonas* spp, and *Candida albicans*.¹¹

One disadvantage of partial decontamination is the risk of anaerobic infection. Our patients did not develop an anaerobic infection, although four (cases 1, 4, 5, and 6) suffered from graft-versus-host disease with partial desquamation of the mucosa of the gut. There is probably less risk of anaerobic infection when no aerobic potential pathogens are present in the gut to lower the redox potential ratio in tissues.

Although infection remains a real problem in patients undergoing bone-marrow transplantation, chemotherapy, or any other treatment that interferes dramatically with the host's resistance to infection, we saw a low morbidity and no mortality due to bacterial or fungal infection in these patients during partial decontamination. Systemic antibiotic treatment and transfusions of granulocytes were seldom necessary. Strict isolation was unnecessary for some patients, and semi-isolation proved adequate.

Partial antibiotic decontamination merits further trial as a technique for preventing infection in patients with acquired or congenital immunodepression. It may be valuable even when facilities for strict isolation are not available.

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