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Prevention of Infection and Graft-versus-Host Disease by Suppression of Intestinal Microflora in Children Treated with Allogeneic Bone Marrow Transplantation

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The effect of suppression with antimicrobial agents of the intestinal microflora of paediatric bone marrow graft recipients on severe bacterial and fungal infections and on moderate to severe acute graft-versus-host disease was studied retrospectively. Data on 65 cases of bone marrow transplantation for either severe bone marrow failure or leukaemia, performed in a strict protective environment with either complete or selective gastrointestinal decontamination, were evaluated. All bone marrow grafts were from HLA-identical siblings and were not depleted of T-lymphocytes. Twenty percent of the recipients had one or more episodes of septicaemia during the granulocytopenic period after transplantation, mostly due to gram-positive bacteria. Only five children died due to infection, in each case caused by a microorganism originating from the endogenous flora. Complete gastrointestinal decontamination was superior to selective gastrointestinal decontamination in preventing infectious complications ($p < 0.001$). The same was the case for the prevention of acute graft-versus-host disease of grade II or higher, which was observed in 7 of 40 (17.5 %) completely decontaminated children versus 9 of 18 (50 %) selectively decontaminated children evaluable for graft-versus-host disease ($p < 0.01$). It is concluded that complete gastrointestinal decontamination in a strict protective environment is a feasible and very effective method for preventing severe infections and acute graft-versus-host disease after allogeneic bone marrow transplantation in children and adolescents; it resulted in a low transplantation-related mortality of 26 % and a good quality of survival in 69 % of the graft recipients.

Bacterial and fungal infections, and acute and chronic graft-versus-host disease (GvHD) are major causes of morbidity and mortality following allogeneic bone marrow transplantation (BMT). This holds also for children and adolescents as recipients of bone marrow allografts. Severe bacterial and fungal infections in the early post-BMT period were reported to occur in 20–50 % of BMT recipients grafted before 1980, leading to death in about half of them (1, 2). Recent retrospective evaluations demonstrated a frequency of severe bacterial and fungal infection in 15–25 % of graft recipients, contributing to the death in about half of them (2). Data obtained from the European Bone Marrow Transplant Registry for children (age < 15 years) grafted for severe aplastic anaemia between 1970 and 1980 (n = 71; comparable with the severe aplastic anaemia patients of this study) gave an overall transplantation-related mortality rate of 50 %, and a mortality rate due to bacterial or fungal infection of 14 % (A. Locasciulli, personal communication). The mortality rate due to transplantation-related complications for children and adolescents (age < 20 years) grafted for leukaemia and registered in

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the European Bone Marrow Transplant Registry from 1980 until 1987 (n = 692; comparable with the leukaemia patients of this study) was 26 % overall, and 11 % due to bacterial or fungal infection as the major cause (J. Hermans, personal communication). Forty percent of children and adolescents (age < 20 years) suffered from moderate to severe (≥ grade II) acute GvHD (3), and 40 % from chronic GvHD (4) after allogeneic BMT.

For the control of infections, protective environments and the suppression of the intestinal microflora of the host by gastrointestinal decontamination (GID) have been used. These measures were demonstrated to be effective for the prevention of severe infections, especially with gram-negative facultative anaerobic intestinal bacteria, yeasts and fungi (5–13). Considerable experimental evidence exists indicating that GID mitigates GvHD (14–18). In man, the possible effect of GID on GvHD is controversial: several studies reported a reduction of the incidence of acute GvHD after allogeneic BMT (19, 20, 21). However, such an effect was not observed in some other studies (22, 23, 24). Here we report on a retrospective evaluation of the efficacy of GID in a protective environment for the prevention of severe bacterial and fungal infections and of GvHD in 65 children and adolescents, grafted consecutively for either severe bone marrow failure or leukaemia. Part of this retrospective evaluation in 24 patients has been briefly reported previously (25).

Patients and Methods

Graft Recipients and Transplant Procedures. All 65 children and adolescents grafted consecutively in a period of 14 years (between 1971 and 1986) for either severe bone marrow failure (n = 29) or leukaemia (n = 36) were included in this retrospective evaluation. All patients received full bone marrow grafts from HLA genotypically identical siblings following the usual pre-treatment regimens. Children with severe aplastic anaemia and Fanconi's anaemia received either cyclophosphamide (50 mg/kg/day × 4) alone or cyclophosphamide (50 mg/kg/day × 4) plus procarbazine (15 mg/kg/day × 3) and rabbit anti-thymocyte globulin (2 mg/kg/day × 3), or cyclophosphamide (50 mg/kg/day × 4) plus total body irradiation (TBI, 4 Gy, high dose rate, in one session). For myelodysplastic syndrome and leukaemia, the conditioning regimen consisted of cyclophosphamide (60 mg/kg/day × 2) plus TBI (7–8 Gy, high dose rate, in one session, depending on age). Fifty-nine recipients received methotrexate for GvHD-prophylaxis until day 102 after BMT (26) and six received cyclosporin-A (2 mg/kg/day in continuous i.v. infusion during ≥ 2 months, followed by 6 mg/kg/ day orally until at least 6 months after BMT).

Gnotobiotic Measures and Surveillance Cultures. The patients were maintained in a strict protective environment, i. e. in laminar down-flow isolators (27), using aseptic nursing techniques and sterilization of food, beverages and all other items brought into the isolator. Forty-four recipients received high doses of non-absorbable antimicrobial drugs for complete GID, and 21 underwent selective GID with either absorbable antimicrobial drugs or low doses of non-absorbable antimicrobial drugs (Table 1). The two modes of GID were used in succession: 1971–1977 complete GID, 1977–1982 selective GID and 1982–1986 complete GID. Decontamination was started one week or more before the date of BMT and was given for at least a total of 40 days after BMT, according to experimental data (28). After discontinuation of GID, the recipients were recontaminated by oral lavage of a mixture of lyophylized cultures of *Lactobacillus acidophilus, Bifidobacterium bifidum* and *Streptococcus thermophilus* (Biogarde, Sanofi-Bio-Industries, FRG) and an anaerobic human-derived donor flora (29, 30, 31). They left the protective environment at about three months (96 ± 43 days) following BMT, depending on other parameters such as general condition and degree of haematological and immunological recovery. The characteristics of the recipients, subdivided according to the type of GID, are given in Table 2. Neither systemic antimicrobial drugs (except for *Pneumocystis carinii* prophylaxis with co-trimoxazole) nor granulocyte transfusions were used prophylactically in these patients. In individual cases, 5-fluorocytosine was administered when GID for yeasts in the gut had not been successful.

The target microorganisms of GID were all bacteria, yeasts and fungi of the gastrointestinal tract in the case of complete GID, and gram-negative facultative anaerobic bacteria, staphylococci, yeasts and fungi of the gastrointestinal tract in the case of selective GID. Surveillance cultures (performed at the Microbiological Laboratory of the Department of Haematology, Leiden University Hospital) for the target microorganisms, including potentially pathogenic microorganisms (*Pseudomonas aeruginosa, Enterobacteriaceae, Streptococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Candida* spp. and *Aspergillus* spp.) of skin, nose and throat swabs and samples of faeces, were made twice weekly during the whole observation period, i. e. from 7 days before until

Table 1: Antimicrobial agents used for complete and selective gastrointestinal decontamination.

Type of decontamination	Drugs	Daily dosage (mg)
Complete	neomycin	1000[a] 2000[b]
	polymyxin B	1000[a] 2000[b]
	cephaloridin	1000[a] 2000[b]
	amphotericin B	1000[a] 2000[b]
Selective	nalidixic acid[d]	90[c]
	co-trimoxazole[d]	12/60[c]
	polymyxin B	20[c]
	neomycin	15[c]
	amphotericin B	1000[a] 2000[b]

[a]For patients < 2 years of age.
[b]For patients ≥ 2 years of age.
[c]Per kg body weight.
[d]Used only in a limited number of patients.

Table 2: Patient characteristics.

	Decontamination	
	Complete	Selective
Number of patients	44	21
Age (years)		
Range	1–17	< 1–16
Median	10	10
Sex (m/f)	23/21	12/9
Diagnosis		
Severe aplastic anaemia	11	12
Fanconi's anaemia	2	3
Myelodysplastic syndrome	2	1
Acute non-lymphocytic leukaemia	9	5
Acute lymphocytic leukaemia	19	0
Chronic myelocytic leukaemia	1	2

40 days after BMT. Routine culturing of strictly anaerobic bacteria was not performed because it was impossible to process samples soon enough after they were collected due to logistic problems, so that reliable data could not be obtained. Gram stains were made of faecal smears from the samples taken for culturing and investigated microscopically for the diversity and quantity of the faecal microflora and for the possible presence of epithelial cells and white blood cells (32). After discontinuation of GID, surveillance culturing was continued once weekly until termination of protective isolation and discharge from hospital.

Evaluation of Findings. Both complete and selective GID were considered successful when in the period from 7 days before until 40 days after BMT the target-microorganisms could not be isolated from more than two consecutive faecal samples. Episodes of bacteraemia and fungaemia, severe organ infection (such as otitis media, sinusitis, lower respiratory tract infections, urinary tract infections, abscesses) and death from infection were recorded. Severe infections were categorised as sepsis or local infection. Only microbiologically documented infections were taken into consideration. Infectious episodes had to be separated from each other by a period of two days or more with rectal temperatures below 38 °C and negative culture results, or negative x-ray findings in the case of lower respiratory tract infection. The evaluation period for severe infection was from the day of BMT until termination of protective isolation measures and discharge from the isolator. Peripheral blood granulocyte counts, i. e. the sum of band-forms and neutrophilic granulocytes, were determined three times a week for at least 40 days after BMT, and twice weekly thereafter during the whole evaluation period. The rate of recovery of peripheral blood granulocytes, assessed by the time in days after BMT in which the sum of band-forms and neutrophilic granulocytes reached a count of 0.1×10^9/l, 0.5×10^9/l and 1.0×10^9/l, respectively, was also evaluated.

GvHD was diagnosed on the basis of clinical symptoms. The severity of acute GvHD was graded according to Thomas et al. (33). In all cases with GvHD of grade II or higher the diagnosis was confirmed by histological evaluation of biopsies from skin, gut or liver. The severity of chronic GvHD was graded as either limited in the case of single-organ involvement (mostly skin), or extensive in the case of multiple organ involvement. The evaluation period for GvHD was not limited.

For statistical evaluation the t-test was used to test for significance of differences between means. Differences in proportions were tested for their significance with the chi-square test, using the Yates correction for $n < 30$. Survival was studied using actuarial survival curves and tested for possible significant differences by the log-rank test.

Results

Gnotobiotic Measures. According to the criteria used, GID was successful in 12 of 44 children who underwent complete GID (27 %) and in 17 of 21 children who underwent selective GID (81 %). The results of microbiological surveillance cultures in the different groups of patients are given in Table 3. From these data it can also be seen that unsuccessful selective GID was only due to persistence of yeasts (*Candida* spp.) in contrast to failure of complete GID, which was due to bacteria as well.

The recovery of granulopoiesis, indicated by the counts of band-forms and neutrophils in the peripheral blood after BMT, is shown in Figure 1. Six children were excluded from this evaluation, including four patients who died early after BMT, before day + 16, and two patients without engraftment. The period after which peripheral blood granulocytes reached

Table 3: Results of microbiological cultures of faecal samples in children with gastrointestinal decontamination (from day – 7 until day + 40 after allogeneic BMT).

Type of GID	Success of GID	(n)	No. of samples cultured (median and range)	Positive culture (%)		
				Gram-negative bacteria	Gram-positive bacteria	Yeasts
Complete	yes:	12	134 (12; 4–17)	0.75	1.49	0.75
	no:	32	416 (13; 7–28)	15.87	20.19	37.98
Selective	yes:	17	197 (12; 3–16)	3.05	NA	2.54
	no:	4	47 (12; 9–13)	2.13	NA	70.21

NA: not applicable.

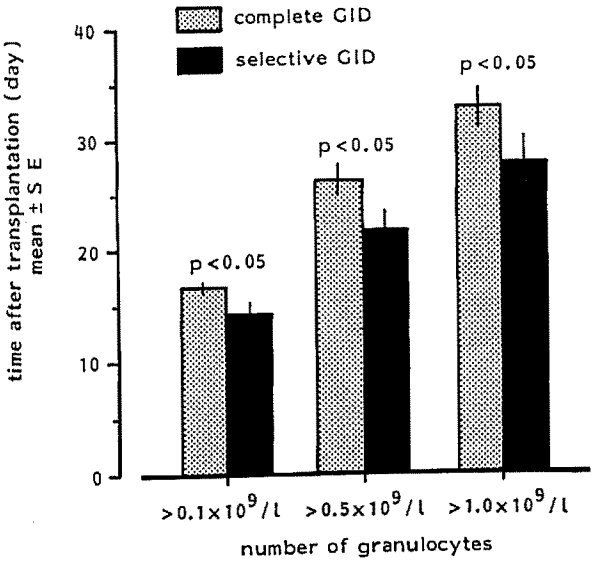

Figure 1: Recovery of granulocytes after allogeneic BMT in children with complete (n = 41) and selective (n = 18) gastrointestinal decontamination.

counts of 0.1×10^9/l, 0.5×10^9/l and 1.0×10^9/l was significantly delayed in the group of completely decontaminated patients compared with selectively decontaminated patients.

Infections with Bacteria, Yeasts, and Fungi. Episodes of microbiologically documented severe infection in 64 graft recipients are given in Table 4 (1 patient who died of cardiac failure on the day after BMT was excluded). The number of infectious episodes was significantly lower in the group of completely decontaminated children ($p < 0.001$). The distribution of the infections in the patients is given in Table 5. In both groups of completely and selectively decontaminated patients, 10 patients had 1 infection, while in each group 1 patient had 2 infectious episodes. In addition, 1 selectively decontaminated patient had 4 different infectious episodes. The total number of patients with 1 or more infectious episodes was 11 of 44 completely decontaminated patients, and 12 of 20 selectively decontaminated patients ($p < 0.05$). None of the patients developed infections with newly acquired gram-negative bacteria, as proved by biotyping (API-System, France). Infections with gram-negative bacteria were only observed before 1980 in patients with severe aplastic anaemia who had a history of infection with the same microorganism before BMT. The infections, leading to the death of five patients, were caused by endogenous microorganisms present in the patient before the start of protective isolation and decontamination. The fatal infections, caused by *Staphylococcus aureus, Klebsiella pneumoniae* and *Candida albicans*, occurred in children grafted for severe aplastic anaemia who either had long-lasting severe granulocytopenia before BMT or a bone marrow graft which did not take. The fatal infection caused by *Aspergillus fumigatus* was in the form

Table 4: Episodes of severe infections with bacteria, yeasts and fungi (from the day of BMT until the day of discharge out of the isolator).

Type of GID	Number of episodes	Causative microorganisms (n)	Sepsis	Local infection	Fatal infection
Complete (n = 44)	12[a]	*Enterobacter* spp. (1)		1	
		Staphylococcus aureus (3)	3		1
		Staphylococcus epidermidis (4)	4		
		α-haemolytic streptococci (1)	1		(1)[c]
		Candida albicans (1)?	(1)[d]		
		Aspergillus fumigatus (2)		2	1
		Total	8 (+ 1)	3	2 (+ 1)
Selective (n = 20)	16[b]	*Klebsiella pneumoniae* (1)	1		1
		Pseudomonas aeruginosa (1)	1		
		Staphylococcus epidermidis (8)	6]1[e]	1	
		Streptococcus faecalis (2)	1		
		α-haemolytic streptococci (4)	4		1
		Candida albicans (1)	1		1
		Total	15	1	3

a–b: $p < 0.001$.
[c]Death caused by other factors in association with α-haemolytic streptococcal sepsis.
[d]Only demonstration of *C. albicans* antigen in blood.
[e]One episode of sepsis with two microorganisms (*S. epidermidis* + *S. faecalis*).

of a bronchial aspergilloma in a boy grafted for acute lymphocytic leukaemia, which led to massive pulmonary bleeding shortly after BMT. On histopathological examination of autopsy material, the aspergilloma was found to have perforated the wall of a bronchial artery. Alpha-haemolytic streptococcal bacteraemia was associated with the fatal outcome in two patients. One child, grafted for juvenile type chronic myelocytic leukaemia, died 16 days after BMT of acute pulmonary edema and bleeding caused by adult type respiratory distress syndrome in association with the α-haemolytic streptococcal bacteraemia. In the other patient, who was grafted twice for severe aplastic anaemia, α-haemolytic streptococci were isolated from blood cultures during a period of 78 days until the time of death 27 days after the second BMT. At the same time this patient suffered from general wasting and had signs of cardiotoxicity; the graft was not successful.

Most cases of bacteraemia were caused by gram-positive microorganisms, 56 % by staphylococci. The number of skin cultures positive for staphylococci in the period from day − 7 until day + 40 after BMT in completely and selectively decontaminated children and the number of cases of bacteraemia with staphylococci in the same period is given in Table 6. Both the number of positive skin cultures and of bacteraemia cases were significantly higher ($p < 0.001$ and $p < 0.010$ respectively) in selectively decontaminated patients compared with completely decontaminated patients. This finding suggests a causal relation between the two variables. However, in almost all selectively decontaminated patients *Staphylococcus epidermidis* was cultured from the throat, which may well have been the portal of entry for this organism.

Acute and Chronic Graft-versus-Host Disease. The effect of GID on acute and chronic GvHD could be evaluated in 58 and 48 patients, respectively. Seven children were not evaluable for acute GvHD: one was grafted with bone marrow from a syngeneic donor, four patients died before 16 days after BMT, and two patients had a graft which did not take. Ten more patients were not evaluable for chronic GvHD. One patient with severe aplastic anaemia had a late rejection of the graft and autologous recovery, and nine patients died of other causes before day 100 after BMT: one patient of renal failure 59 days after BMT, one of *Toxoplasma gondii* encephalitis 85 days after BMT, one of interstitial pneumonia 90 days after BMT, and in six patients death was due to acute GvHD.

Table 7 summarizes the data on acute GvHD. None of 11 successfully completely decontaminated recipients developed acute GvHD of grade II or higher. The group of 29 children in which complete GID was unsuccessful had less acute GvHD than the group of 14 patients who were selectively decontaminated with success (24 % versus 43 %), although this difference was not statistically significant. The group of com-

Table 5: Distribution of the infections.

Type of GID	Number of patients				Total number of infected patients
	1 infection	2 infections	3 infections	4 infections	
Complete (n = 44)	10	1	–	–	11[a]
Selective (n = 20)	10	1	–	1	12[b]

a–b: $p < 0.05$.

Table 6: Positive skin cultures (staphylococci) and staphylococcal bacteraemia in decontaminated children (from day − 7 until day + 40 after allogeneic BMT).

Type of GID	Number of patients	Number of bacteraemia cases (staphylococci)	Total number of skin cultures	Number of positive skin cultures (staphylococci)
Complete	44	3[a]	566	297[c] (52 %)
Selective	20	6[b]	238	166[d] (70 %)

a–b: $p < 0.010$.
c–d: $p < 0.001$.

pletely decontaminated patients as a whole did significantly better with respect to acute GvHD than the selectively decontaminated group, whether successful or not [(a +b) versus (c + d), c, and d; Table 7]. Significantly less chronic GvHD, especially extensive chronic GvHD, was observed in the completely decontaminated group of patients (Table 8).

The advantage of complete over selective GID can also be assessed by comparing the risk of transplantation-related mortality (Figure 2), as well as the combined risk of transplantation-related mortality and development of severe chronic GvHD (Figure 3) in the two groups of patients. The chances of a good quality of survival are significantly better ($p < 0.05$) for the completely decontaminated bone marrow graft recipients.

Table 7: Effect of the success of gastrointestinal decontamination (GID) on the occurrence of ≥ grade II acute graft-versus-host disease (GvHD).

Number of patients	Type of GID	Success of GID	Number of patients with ≥ grade II acute GvHD
40	complete	yes: 11 no: 29	0^a 7^b
18	selective	yes: 14 no: 4	6^c 3^d

Significant differences: (a+b) – (c+d): $p < 0.01$.
a – c : $p < 0.05$.
a – (c+d): $p < 0.05$.
(a+b) – c : $p < 0.05$.
(a+b) – d : $p < 0.005$.

All other differences: $p > 0.05$ (NS).

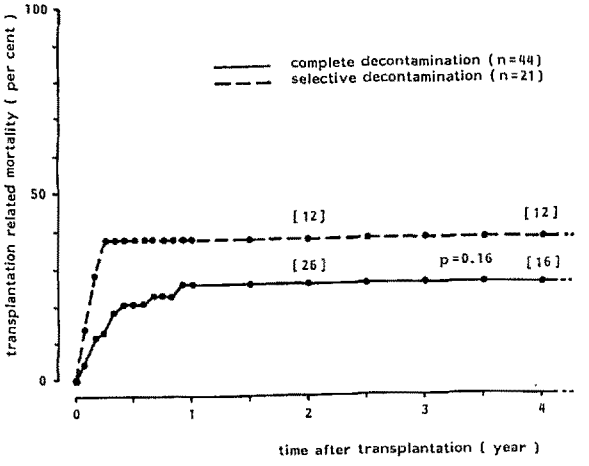

Figure 2: Risk of transplantation-related mortality for completely and selectively decontaminated patients after allogeneic BMT. Number of patients at risk given in brackets.

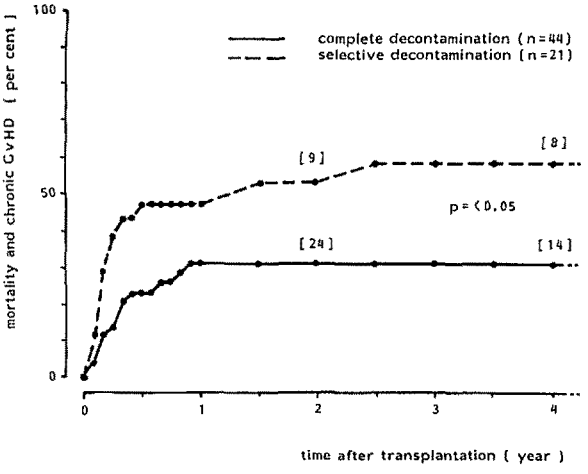

Figure 3: Risk of transplantation-related mortality or development of chronic GvHD for completely and selectively decontaminated patients after allogeneic BMT. Number of patients at risk given in brackets.

Table 8: The success of gastrointestinal decontamination (GID) on the occurrence of chronic graft-versus-host disease (C-GvHD).

Number of patients	Type of GID	Success of GID	Number of patients with C-GvHD		
			All	Limited degree	Extensive degree
35	complete	yes: 10 no: 25	2^a 5^b	2^e 2^f	0^i 3^j
13	selective	yes: 11 no: 2	5^c 2^d	2^g 1^h	3^k 1^l

Significant differences: (a+b) – (c+d): $p < 0.05$.
(a+b) – c : $p < 0.05$.
(a+b) – d : $p < 0.05$.
(i+j) – (k+l): $p < 0.05$.

All other differences: $p > 0.05$ (NS).

Discussion

In this retrospective study the efficacy of gnotobiotic measures in preventing severe infections and GvHD in children treated with allogeneic non-T-cell depleted BMT was evaluated. The main difference between the two study groups (complete and selective decontamination) with regard to number and entry characteristics was the fact that 19 patients with acute lymphocytic leukaemia entered the study after 1982 and consequently all underwent complete decontamination. For the sake of clarity, the effect of complete and selective GID, both applied in a strict protective environment, on infectious complications and on GvHD will be discussed separately and will be correlated with success or failure of GID.

In order to reach valid statements regarding the possible efficacy of gnotobiotic measures in bone marrow graft recipients, the criteria for successful GID should be defined precisely. Although several publications deal with GID in BMT (2, 12, 19, 20, 21, 24, 34–40), only Skinhøj and colleagues (24) present a clear definition of successful complete GID as the presence of bacteria or yeasts and fungi in less than three of the bi-weekly surveillance cultures over a median period of 28 days. The success rate of complete GID was 17 % (11/65) in their study (24), which is of the same order as that obtained in our study of 27 % (12/44). Failure in both studies was mainly due to persistence of yeasts. In earlier studies it was shown that the suppression of yeasts which persisted in the gastrointestinal tract during complete GID could be achieved by recolonization of the gut with anaerobic donor flora (29). This illustrates the competition between microorganisms in the gastrointestinal ecosystem. It may also explain the difference in success rate between selective (81 %) and complete (27 %) GID. More recently, we added i. v. 5-flucytosine to the complete decontamination regimen if yeasts persisted in cultures of either throat swabs or faecal samples shortly after suppression of bacteria. An additional suppressive effect on yeasts was observed in several children (unpublished data) without an adverse effect on haematological recovery. This is in accordance with the reported finding of in vitro colony formation of haemopoietic progenitor cells following administration of 5-flucytosine, in contrast to the suppressive effect of 5-fluorouracil, a metabolite of 5-flucytosine produced by gut bacteria (M. Kissling, 5th International Symposium on Infections in the Immunocompromised Host, Noordwijkerhout, 1988, Abstract no. 189).

Although not determined exactly, the rate of compliance in children given oral antimicrobial drugs for GID was superior in the selectively decontaminated group. In our experience compliance decreased with increasing age of the recipient. In one case poor compliance resulted in fatal *Staphylococcus aureus* infection in a completely decontaminated patient with severe aplastic anaemia who did not have an established graft.

The frequency of bacteraemia and candidaemia in the groups studied was comparable to the findings reported by other investigators, who used some form of protective environment and GID (1, 2, 39, 40): 13 of 64 patients (20 %) experienced a total of 15 episodes of septicaemia during the first month after BMT, i. e. during the neutropenic period. Six patients had a total of eight septicaemia episodes (once with two bacteria simultaneously) more than one month after BMT. In one child *Candida albicans* antigen was demonstrated in the blood at the time of BMT; this could not be confirmed microbiologically. Most septicaemia episodes (20/23, 87 %) were due to gram-positive bacteria. The five fatal infections were all caused by endogenous microorganisms. A case of α-haemolytic streptococcal septicaemia associated with pulmonary capillary leakage and intra-alveolar bleeding was seen in one child shortly after BMT and led to death. This has recently been observed by ourselves and others (41, 42) as a severe and often lethal complication following intensive cytoreductive therapy for haematological malignancies. Coverage with antimicrobial agents active against gram-positive bacteria seems indicated at the first sign of sepsis early after BMT. All in all it may be concluded that the gnotobiotic measures were highly effective in preventing severe infections; complete GID in strict protective isolation was superior to selective GID (Tables 4 and 5).

With regard to the effect of gnotobiotic measures on the prevention of GvHD, this study confirmed our previous finding that complete elimination of the gastrointestinal microflora strongly reduces the incidence of moderately severe and severe acute GvHD (25). It should be emphasized that experimental data and data obtained in our clinical study indicate that GID mitigates GvHD, and not reverse isolation. The most plausible explanation for this finding is that in the case of prolonged elimination of these microorganisms, grafted precursor T-lymphocytes are not activated and clonally expanded by substances (e. g. endotoxins and/or peptidoglycans) from these microorganisms or by antigens on these microorganisms, possibly shared for instance by the gut epithelium of the host. The discrepancy between our findings and those of others (22, 23, 24), who reported no influence of gnotobiotic measures, may be due to either less strict criteria used to define the

success of GID or to too short a period of actual suppression of the gut microflora. In mice, an effect of GID on GvHD has only been observed when the gastrointestinal microflora is eliminated from 10 days before (R. L. Truitt, 17th Annual Meeting of the Association for Gnotobiotics, New York, 1979, Abstract no. 8) until about 40 days after BMT (28).

From Table 7 it is obvious that complete GID was superior to selective GID in preventing acute GvHD of grade II or higher. In the latter treatment group the percentage of children developing this disease was 50 % for the whole group and 43 % for the successfully selectively decontaminated children (Table 7). This agrees with the incidence of 45 % seen in 2,036 recipients of HLA-identical sibling bone marrow transplants analyzed by the International Bone Marrow Transplant Registry (3). None of the 11 successfully completely decontaminated children developed acute GvHD of grade II or higher, while this disease was observed in 7 of 29 (24 %) unsuccessfully completely decontaminated children. The difference between these two groups was not statistically significant, the numbers being limited. The group of (successfully and unsuccessfully) completely decontaminated patients developed significantly ($p < 0.01$) less often acute GvHD of grade II or higher than did the group of (successfully and unsuccessfully) selectively decontaminated patients. We conclude from these two observations that complete GID is to be preferred for the prevention of acute GvHD after allogeneic BMT despite the fact that the success rate of selective GID is higher. Recent experimental data support this conclusion, it having been shown in a murine transplantation model that the anaerobic microflora may play a major role in eliciting acute GvHD after allogeneic BMT (43). Although not all our patients received identical GvHD prophylaxis (59 received methotrexate and 6 cyclosporin-A), this cannot have influenced our results, since an evaluation in 179 leukaemia patients revealed no difference between these two agents with regard to the prevention of acute GvHD (44). The results shown in Table 8 also suggest a beneficial effect of complete GID with respect to development of chronic GvHD. However, it is more likely that this effect is secondary to the prevention of acute GvHD (45).

An additional observation was that the recovery of granulopoiesis, as assessed by the granulocyte counts in peripheral blood, was significantly delayed in completely decontaminated patients compared with selectively decontaminated patients. A similar observation has been made in decontaminated dogs (46) and monkeys (47). The most plausible explanation for this is the absence of (antigenic) microbial stimuli or a decreased rate of release of cytokines (interleukins and growth and differentiation factors) in cases of complete suppression of the gut microflora. From the results of endotoxin determinations in some of these patients (48), it can be concluded that selective GID leaves (anaerobic) bacteria in the gastrointestinal tract which are capable of producing endotoxins.

The ultimate goal of gnotobiotic measures, as applied in this study, is to obtain a higher survival rate with a good quality of life by preventing some major transplantation-related complications. As can be seen in Figure 3, the cumulative rate of these complications was significantly lower in children in the complete GID group than in children in the selective GID group.

Psychosocial studies of large groups of infants and children treated in our department by BMT in strict protective isolation indicated that apart from a temporary feeling of distress almost exclusively present in children above the age of 13 years, there were neither immediate nor late adverse effects of this type of treatment, in comparison with more conventional types of intensive haemato-oncological treatment (49, 50).

In our opinion the effectiveness of gnotobiotic measures (i. e. complete GID and protective isolation) more than counterbalances the costs and the inconvenience of these procedures. This is in contradiction with assertions made by others (39, 40, 51) that gnotobiotic measures are distressing for patients, inconvenient for the staff and expensive. In an extensive cost-benefit analysis of BMT performed by an independent team for the Health Council of The Netherlands (52), it was found that the expenses for materials for GID comprised less than 5 % of the total expenses for material during the admission period of patients; major expenses were laboratory tests (about 40 %), blood transfusion products, antibiotics for systemic therapy, immunosuppressive drugs and parenteral nutrition (each about 13 %). From these figures it can be concluded that gnotobiotic measures are only a minor part of the total costs of BMT. By preventing infectious complications and GvHD, such measures save the expense of systemic antimicrobial treatment, transfusions of granulocytes and other blood products, immunosuppressive drugs and prolonged parenteral nutrition.

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