THE POSSIBLE ROLE OF THE ID-NETWORK IN THE DEVELOPMENT OF LATE ONSET GRAFT-VERSUS-HOST DISEASE AFTER BONE MARROW TRANSPLANTATION: IMPORTANCE OF THE MICROFLORA OF THE BONE MARROW RECIPIENT

PETER J. HEIDT[1] and DIRK VAN DER WAAIJ[2]

[1]Department of Animal Science, Biomedical Primate Research Centre, Rijswijk, and [2]Hoge Hereweg 50, Glimmen, The Netherlands

SUMMARY

One of the major complications of allogeneic bone marrow transplantation (BMT) is graft-versus-host disease (GvHD), which is caused by donor type lymphocytes which react against the recipient's tissues.

An important factor which influences GvHD is the recipient's gastrointestinal microflora. This was originally observed in gnotobiotic mice. Infusion of 10^7 H-2 incompatible bone marrow cells into lethally irradiated (9.0 Gy X-rays) conventional mice results in a late onset type GvHD which causes the death of the majority of the recipients during the first two months after BMT. This mortality can be completely prevented if the recipients are germfree mice, or when they are conventional animals which have been subjected to complete gastrointestinal decontamination.

Donor (C57Bl/Rij) and recipient (C3H/Law) mice with different defined microfloras were obtained by using offspring from germfree C3H/Law mothers that were contaminated with the different floras or by using those C3H/Law females to foster hysterectomy-derived C57Bl/Rij new-borns. Studies using these donor and recipient mice resulted in significantly different mortality rates due to GvHD. This observation can be explained by the hypothesis that during gestation and fostering, the developing immune system of new-born animals is modulated by the "experienced" immune system of the dam, resulting in immunological tolerance to flora-components of the (foster-) mothers.

INTRODUCTION

Allogeneic bone marrow transplantation (BMT) is an accepted treatment for many fatal diseases of the haemopoietic system, among them severe aplastic anaemia and leukaemia. Furthermore, patients suffering from fatal hereditary diseases that are associated with a dysfunction of the lymphoid system, like severe combined immunodeficiency, and patients with inherited severe metabolic disorders are being treated with bone marrow grafts.

One of the major complications of allogeneic BMT is graft-versus-host disease (GvHD), which is caused by donor type lymphocytes which react against the recipient's tissues. According to an evaluation of data from 2036 recipients of HLA identical sibling bone marrow transplants reported to the International

Table 1: Composition of the SPF- and Houston-flora

SPF-flora (SPF)	Houston-flora (HF)
Anaerobic microflora:	**Anaerobic microflora:**
Not defined	Not defined
Aerobic microflora:	**Aerobic microflora:**
Streptococcus faecalis (7173711♣)	*Streptococcus faecium* (7355510♣)
	Streptococcus faecium (7317550♣)
Staphylococcus aureus (6726153♦)	*Staphylococcus xylosus* (6736552♦)
Staphylococcus epidermidis (6706133♦)	*Staphylococcus haemolyticus* (6632171♦)
Escherichia coli (5144572♠)	*Escherichia coli* (5144572♠)
	Escherichia coli (5144532♠)
	Proteus mirabilis (0536000♠)
	Proteus mirabilis (0534000♠)
	Pasteurella pneumotropica (1220000♥)[1]

♣ : Biotype (API 20 Strep; API System, Montalieu-Vercieu, France)
♦ : Biotype (API Staph)
♠ : Biotype (API 20 E)
♥ : Biotype (API 20 NE)
[1] : Isolated from nasal washings only

Bone Marrow Transplant Registry, moderate to severe GvHD occurred in about 45% of these patients. In 48% of them, GvHD was related to their death (*Gale* et al., 1987). The severity of GvHD is influenced by several factors, which include the degree of immuno-genetic disparity (*Uphoff* and *Law*, 1958) the number of cells grafted (*van Bekkum*, 1964), the number of T-lymphocytes present in the graft (*van Bekkum*, 1964; 1972), the donor's sex (*Gale* et al., 1987) and the age of the recipient (*Gale* et al., 1987).

MICROFLORA AND GRAFT-VERSUS-HOST DISEASE

Another important factor influencing GvHD is the gastrointestinal microflora of the recipient. This was originally observed in gnotobiotic mice. Infusion of 10^7 H-2 incompatible bone marrow cells into lethally irradiated (9.0 Gy X-rays) conventional mice results in a late onset type GvHD which does not give rise to symptoms until about three weeks after BMT. This disease kills the majority of the recipients during the next two months but those that survive for more than three months seem to have recovered (*van Bekkum* and *de Vries*, 1967; *van Bekkum* et al., 1974). Mortality at-tributable to this type of GvHD can be completely prevented if the recipients are germfree mice (*van Bekkum* et al., 1974; *Jones* et al., 1971; *Truitt*, 1978) or when they are conventional animals which have been subjected to complete gastrointestinal decontamination by means of orally administered non absorbable antibiotics prior to transplantation (*Truitt*, 1978; *Heit* et al., 1973). These findings in mice suggest that not only histoincompatibility determines the occurrence and severity of GvHD, but that microflora-related factors also are of major importance.
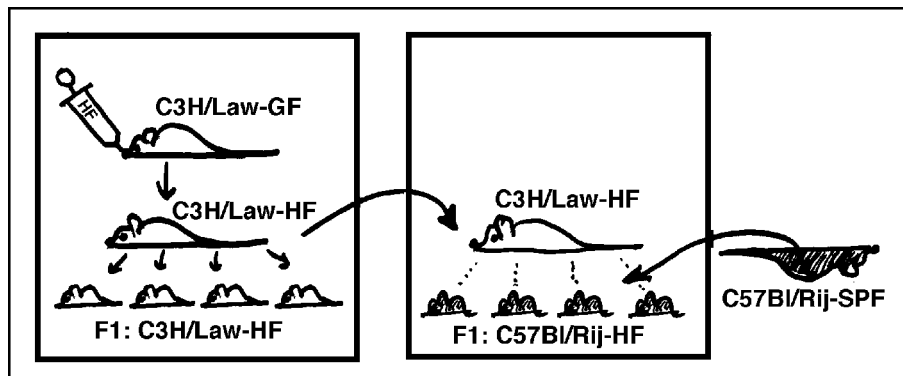
Figure 1: Production of F1 C3H/Law-Hf and F1 C57Bl/Rij-Hf recipient and donor mice.

To study the mechanism, which underlies the influence of the gastrointestinal microflora-components on GvHD, H-2 different donor (C57BL/Rij) and recipient (C3H/Law) mice with a specified pathogen free (SPF) and a conventional microflora were employed (*Heidt*, 1989). For this purpose a conventional microflora was imported from the M.D. Anderson Cancer Institute, Houston, TX, USA, called "Houston flora" (HF), since at that time only SPF animals were being bred in our institute. The composition of the SPF flora and this HF is given in Table 1.

HF bearing C3H/Law mice were obtained by associating germfree C3H/Law breeding pairs with the conventional (HF) flora (Figure 1). Their offspring (C3H/Law-HF) was used as donor or recipient for the different experimental groups. HF bearing C57BL/Rij mice were obtained by foster nursing caesarean derived C57BL/Rij new-borns by C3H/Law-HF mothers (Figure 1).

Before entering the experiment, all mice were kept in Trexler type plastic film isolators to prevent undue association with any other microorganisms. During the experiments, all recipients were housed under conditions of strict reverse isolation in a laminar cross flow isolator to prevent contamination of the animals with any new microorganisms (*van der Waaij* and *Andreas*, 1971). The animals received autoclaved (10 min., 134°C) AM-II food pellets (Hope Farms B.V., Woerden, The Netherlands) and acidified (pH 2.8) sterile drinking water.

According to the microbiological status of the donors and the recipients, there were four different experimental groups. They were: C3H/Law-HF recipients of C57BL/Rij-SPF donor bone marrow (SPF→HF), C3H/Law-HF recipients of C57BL/Rij-HF donor bone marrow (HF→HF), C3H/Law-SPF recipients of C57BL/Rij-SPF donor bone marrow (SPF→SPF), and C3H/Law-SPF recipients of C57BL/Rij-HF donor bone marrow (HF→SPF).

The recipients were lethally (9 Gy) irradiated as a conditioning for BMT. The next day, they were injected i.v. with 10^7 bone marrow cells from C57BL/Rij donor mice. Irradiation of the mice and transplantation of the bone marrow cells were also performed under conditions of strict reverse isolation.

No significant mortality from GvHD occurred in HF→HF, SPF→SPF and HF→SPF recipients (p>0.10), but the mortality of SPF→HF recipients was 80% (Figure 2).
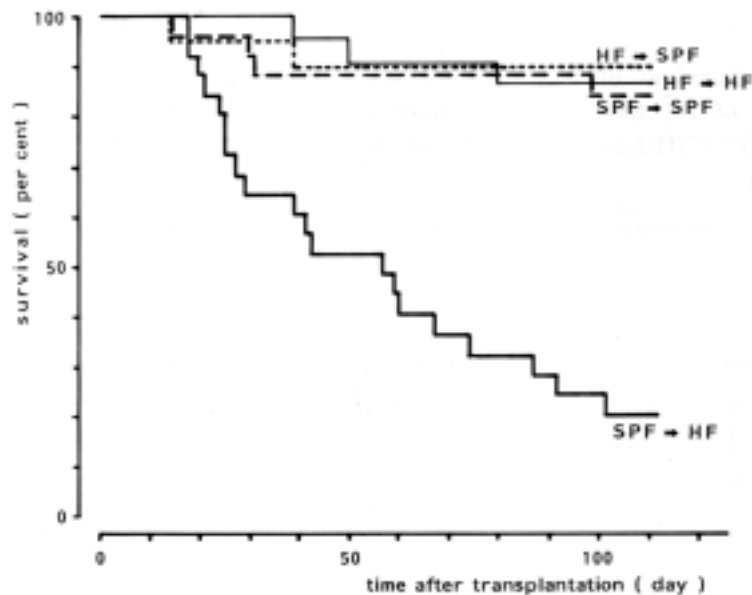
Figure 2: Survival of irradiated (9Gy) C3H/Law recipients of 10^7 C57Bl/Rij bone marrow cells in the different experimental groups (SPF→HF, HF→HF, SPF→SPF, and HF→SPF).

HYPOTHESIS

The difference in survival of the different experimental groups can be explained by a modulating influence of the developing immune system by the "experienced" immune system of the dam (see Figure 3). The ontogenesis of the immune system of these offsprings may have been modulated during gestation by the immune system of their natural mothers. After delivery, the immune system of their foster mothers (the newborns being transferred immediately after "birth") may have additionally provided "immunologic information about their intestinal microflora". As a result, these foster nursed (HF) offspring may have had a double (suppressive) immunomodulation regarding the SPF microflora as well as to the HF microflora.

During pregnancy, clonal suppression/deletion, regarding HF-components of the mother may have occurred in the foetuses. After birth, those new-borns which became physiologically associated with the HF of the dams may not have responded with IgG B-cells as vigorously as their (originally germfree) mothers did when associated with the HF. Some litters in the first generation of HF offspring may, as they were possibly missing the polyspecific IgM clone due to a vigorous (IgG) response of their mother, still have formed IgG. In general however, they may have "learned" to "tolerate" the same (wide) spectrum of meanwhile autochthonous intestinal bacteria as their mothers.

At the time of bone marrow harvesting for transplantation, the bone marrow of SPF-mice may not have contained conventional B- or T-cells. However, immune-cells potentially reactive to the microflora of the HF-recipients may have been present in the donor cells.

The immune system of the HF donor mice may have become tolerated for the HF during lactation by their HF foster mothers. The immune system of the
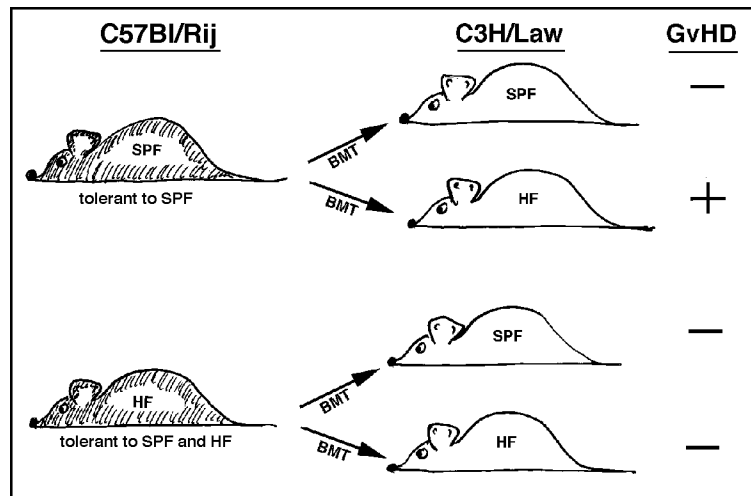
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Figure 3: Influence of donor tolerance to different floras on graft-versus host disease (GvHD) after allogeneic bone marrow transplantation (BMT).

C57Bl/Rij-SPF mice was normally tolerated for the SPF microflora during pregnancy as well as during lactation (the latter may only be important for individuals living in a conventional environment).

Indeed we found only significant GvHD in the SPF→HF donor-recipient combination. No GvHD was seen upon HF→SPF and HF→SPF transplantation.

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