

# Transplantation Immunology

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## Booklet C

### Pretest Correct Answers

3. (C) is correct. One of the major barriers to successful hematopoietic stem cell transplantation is the antigens coded for by the major histocompatibility gene complex. It must be noted that even when a donor is apparently identical to the recipient, typing has only been performed for a relatively small number of genetic markers and there is ample room for differences in those genes that have not been typed. On the positive side, typing includes those markers that have a stronger influence in the outcome of a graft. Identical twins have identical major histocompatibility gene complexes and therefore have identical major histocompatibility complex molecules and they are the ideal source of bone marrow for a transplant. Histocompatible siblings of the same sex are the next best choice, because they are likely to share many other gene markers that have not been typed. Histocompatible siblings of the opposite sex, on the other hand, are incompatible at the H-Y gene system. Individuals such as a parent, first cousin, or unrelated individual, are not likely to be perfect matches and therefore are not ideal donors for hematopoietic stem cells.
  
6. (D) is correct. Clearly, patients who have undergone the required combination of chemotherapy and radiotherapy prior to receiving the stem cell transplant are at increased risk for infection and sometimes must be hospitalized in special clean rooms so as to minimize the chances of exposure to infectious agents. In addition, the hematopoietic stem cell transplant may often contain immunologically competent T lymphocytes, which may attack the recipient's cells causing GVHD. It is known that there is an increased risk of cancer in recipients of hematopoietic stem cell transplants. This risk is thought to be associated with the chemotherapy and radiotherapy which these patients receive prior to receiving the transplants. However, the malignancies almost always originate in host tissues, not from grafted cells. There are some reported cases of donor-derived leukemias, however, although the etiology is not entirely clear. Donor-derived leukemia may result from transfer of an oncogenic clone, indirect injury from residual chemotherapy and radiation damage, abnormal immune surveillance, and/or altered marrow stroma and microenvironment. Finally, not every hematopoietic stem cell transplant is successful; some of these transplants fail to thrive and repopulate the bone marrow. Others are rejected by the host's immune system.
  
10. (A) is correct. The greatest risk associated with being a bone donor marrow is anemia. Typical volume for a bone marrow harvest is around 1300 cc or more, which is about 2-3 times more than a blood donation. All donors are given iron supplements for a month post-harvest, and about half of them require a transfusion. Anemia is less of an issue for peripheral blood stem cell donors, but they may nevertheless have anemia and thrombocytopenia associated with leukopheresis. Infection is possible but rare in the donors. Donors are carefully evaluated for their health and are typically able to regenerate and replace the marrow in several weeks. Bone marrow failure has not been recorded to occur. Transmission of leukemia is not possible, because there is no previous contact of the needle used to harvest the bone marrow with the patient's tissues. Lymphadenopathy has not been reported.

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## Introduction

Thousands of transplants of organs, tissues, and cells are performed throughout the world annually. Transplantation of bone marrow cells or hematopoietic stem cell transplantation (*HSCT*) is performed to correct failures in the production of red or white cells, to repair immune deficiencies, and as a means of replacing malignant cells which have taken over the bone marrow. The major barrier to organ, tissue, and cell transplantation in humans is the immune response to the graft or, in the case of HSCT, the immune response of the graft against the recipient.

This POPS leads to a discussion on the indications and problems associated with HSCT or allogeneic bone marrow transplantation (ABMT) in patients with leukemia. Approximately 70% of the patients undergoing ABMT for the treatment of certain types of leukemia live at least five years. A major barrier to the success of HSCT is the fact that the patient's immune system can reject the donor stem cells or that lymphocytes in the HSCT will attack (reject) the recipient. Donor selection is very important in order to prevent or minimize immune reactions of host against graft and graft against host. It is important to find the best possible histocompatibility match between the donor and the patient.

There are at least six genetic loci in the major histocompatibility complex (MHC), located on chromosome 6, which code for HLA antigens. Each of these six genes is polymorphic meaning that there are many different versions of each gene which differ from one another by a different sequence of nucleotides. Each of these gene variants, called **alleles**, codes for an antigenically different HLA antigen. In the human population there are many, many thousands of different MHC gene combinations or **genotypes**, as they are called. Faced with this huge variability, it is very difficult, although not impossible, to find two human beings who have identical HLA antigens by searching in the population at random. Since HLA antigens are genetically determined, it is often possible to find individuals who are HLA identical, or matched, within a family.

The HLA antigens that receive the most attention for the purposes of transplantation are HLA-A, HLA-B and HLA-DR.

Since each person carries a chromosome six of maternal and paternal origin there are two copies of each HLA gene all of which are expressed (co-dominant expression). Therefore, a "match" typically refers to a "*six-antigen match*" (2 HLA-A, 2 HLA-B, and 2 HLA-DR). Other antigens are also important, including HLA-C and, to a lesser degree, HLA-DQ. Consequently, an 8/8 match refers to an eight-antigen match (2 HLA-A, 2 HLA-B, 2 HLA-DR, and 2 HLA-C), and a 10/10 match includes 2 HLA-DQ. The HLA genes on a particular chromosome 6 constitute a "**haplotype**" (derived from "haploid genotype"). Each person has one maternal and one paternal haplotype based on which of each parent's copies of chromosome 6 was inherited.

The most likely place to find a perfect HLA match between two people is among full-blooded siblings. According to Mendelian inheritance principles, one has a 25% chance of inheriting the same HLA molecules as a sibling, a 25% chance of inheriting none of the same HLA molecules as a sibling and a 50% chance of inheriting half of the same HLA molecules as a sibling. When HSCT is considered, the first thing to do is a "family study" by which the patient and all available siblings and parents are HLA typed. This allows for identification of potential "six-antigen" matches among the siblings and analysis of the segregation patterns of the HLA molecules. The segregation patterns are obtained from determining which of the parental haplotypes are inherited. Each sibling from a given couple should have one haplotype from each parent.

In the example shown below, five of the seven MHC genes of the two families were determined. A number identifies each HLA antigen coded for by an MHC gene. Since we inherit one set of genes from each of our parents, we all have two versions of each MHC gene and thus there are two numbers given for each gene. Because data from both parents and two siblings was available, it was possible to establish the genotypes for the different family members. Discuss the interpretation of the HLA typing data below within the group before turning this page.

### HLA Genotypes

Family Member	HLA Gene	Family 1					Family 2				
		A	B	C	DR	DQ	A	B	C	DR	DQ
Mother		1	5	45	10	21	15	26	40	52	55
		2	6	46	11	22	16	25	41	51	56
Father		3	7	47	12	23	17	27	42	53	57
		4	8	48	13	24	18	28	43	54	58
Child 1		1	5	45	10	21	15	26	40	52	55
		4	8	48	13	24	17	27	42	53	57
Child 2		2	6	46	11	22	15	26	40	52	55
		3	7	47	12	23	17	27	42	53	57

The family members in Family 1 are different from each other and there is no HLA match in the family. However, in Family 2 it is apparent that Child 1 and Child 2 are HLA identical. These examples are shown to make a point about the inheritance of MHC genes and the possibility of finding HLA identical individuals within families. If a sibling or other relative is thought to be a potential match, further testing is done to ensure that the person is the best match possible. This is called "higher-resolution" testing, and is performed by DNA testing utilizing a polymerase-chain reaction (PCR) based assay system.

If a match cannot be found within a patient's family, a search for a matched, an unrelated donor search can be performed through the National Marrow Donor Program (NMDP). The NMDP has a computerized list of people who have agreed to be stem cell donors and their HLA types. Currently, the NMDP has over 4 million people in its registry. Although this sounds like a lot, it must be realized that there are hundreds of thousands to hundreds of millions of different combinations of HLA genes, depending on the number of HLA loci considered. To complicate matters even further, certain HLA antigens are found in greater proportions among members of different racial groups. To put it another way, an African-American patient has a greater chance of matching certain HLA antigens with another African-American. Unfortunately, African-Americans are greatly underrepresented in the NMDP registry thereby decreasing the likelihood of a match for that ethnic group. The same could be said for most minority groups in this country.

Information about joining the NMDP registry can be found on their website, [www.marrow.org](http://www.marrow.org).

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### Data sheet C-1

#### Stem cell isolation

Stem cells can be isolated from the bone marrow or the peripheral blood leukocyte preparation by flow cytometry or using magnetic monoclonal antibodies. The stem cells are separated by their reactivity with CD34 monoclonal antibodies. When using "magnetic" monoclonal CD34 antibodies the stem cells with bound antibodies are retained on a container exposed to a magnetic field. After extensive washing of the container, the magnetic field is removed and CD34<sup>+</sup> stem cells are recovered. Some groups directly inject the isolated stem cells while others expand the CD34<sup>+</sup> stem cells *ex vivo* with cytokine cocktails and then infuse them into the patient intravenously.

However, most groups prefer to use unfractionated CD34<sup>+</sup>-enriched leukocyte preparations (buffy coat) obtained directly from the donor's blood for transplantation, because the patients seem to recover more quickly from the effects of immunoablation with this protocol.

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Approximately 10 days after the HSCT, the patient becomes febrile. Blood cultures are ordered but are negative. The patient was treated with antibiotics and the fever abated. One week after transplantation the patient was given G-CSF to enhance granulocyte production. Fourteen days after HSCT, the patient's white blood count began to increase compared to pretransplant count. The patient was given red blood cell and platelet transfusions twice weekly during the first 10 weeks after HSCT. At one month after HSCT a bone marrow biopsy revealed a relatively vacant marrow (the marrow was hypocellular). Philadelphia chromosome positive cells were not found. At approximately 36 days after HSCT the patient was discharged from the hospital and continued to receive antibiotics plus intravenous gamma globulin (IVIg) and Cyclosporine.

*What do you believe was the cause of this patient's fever?*

*Why were antibiotics prescribed to this patient at the time of discharge?*

*Why was the patient treated with IVIg and cyclosporine?*

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### Data sheet C-3

#### Post-graft evolution (continued)

When the patient developed fever he required antibiotics covering enteric Gram negative and skin Gram-positive organisms, those being the most likely sources of bacteremia. Several antibiotics or combinations of antibiotics can be used, such as a carbapenem (imipenem) by itself or in association with vancomycin, with an extended spectrum penicillin (e.g. piperacillin or ticarcillin), or with an aminoglycoside. At discharge the patient was given ciprofloxacin, to lower the intestinal bacterial load, and IVIg (which contains antibodies to the most common pathogens) to prevent the most prevalent infections in immunosuppressed patients. Cyclosporine was given to prevent the development of a severe form of GVHD.

30 days after discharge the patient came in for a routine clinic follow-up visit and a nurse noticed a rash on the patient's lower back and several lesions on the forearms and palms of the hands, illustrated in figure 1. A biopsy of the affected skin is shown in figure 2.



Figure 1

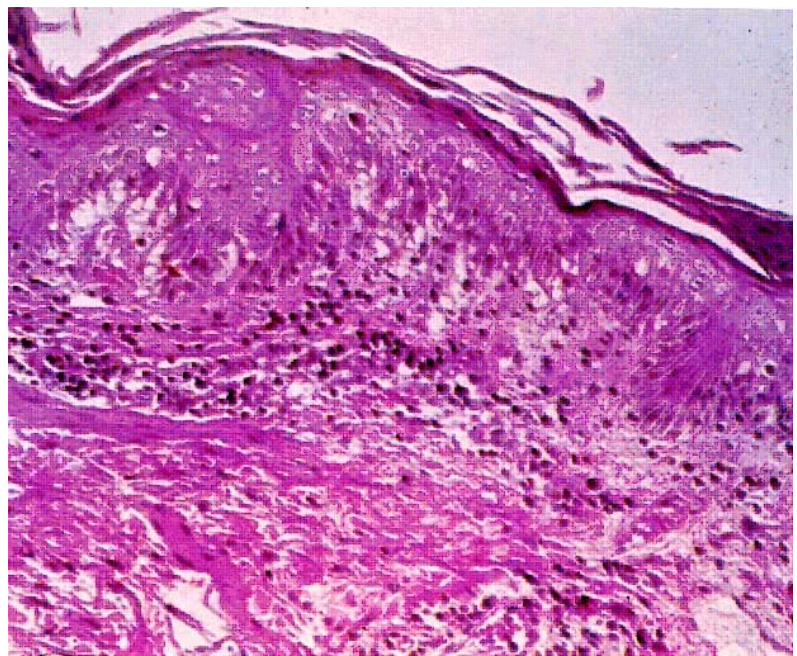


Figure 2

*Photos courtesy of Dr John Maize*

*How do you interpret this biopsy?*

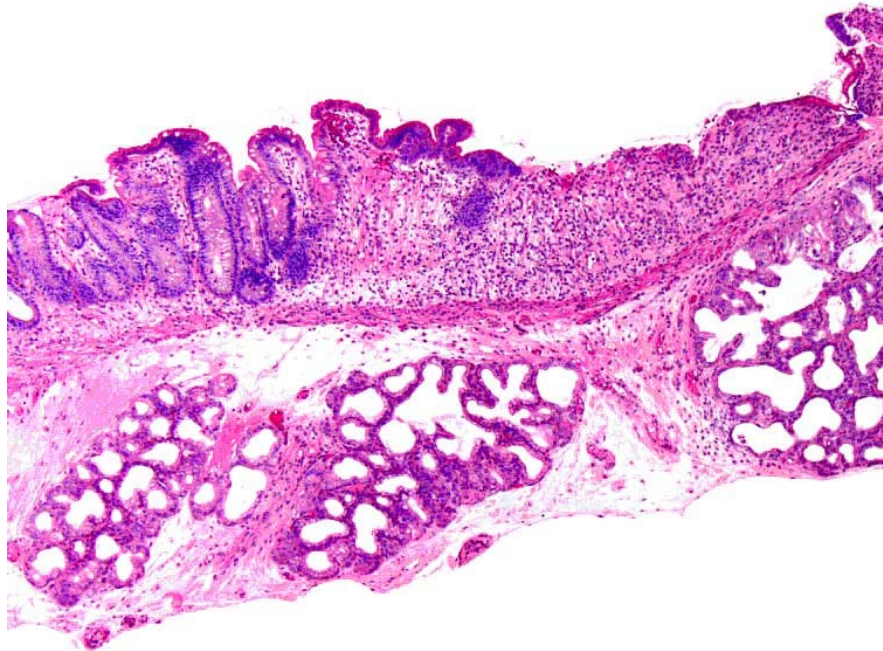
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### Data sheet C-4

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#### Post-graft evolution (continued)

The skin biopsy shows degeneration of the basal layer of the epidermis and a superficial perivascular mononuclear cell infiltrate at the dermo-epidermal junction with invasion of the epidermis by the infiltrating mononuclear cells. High power examination showed apoptotic squamous cells with adjacent lymphocytes in the epidermis. A diagnosis of GVHD was made. In addition to systemic administration of immunosuppressive drugs, topical therapy with corticosteroid cream was prescribed and alleviated the rash. Approximately one month later, the patient came to the clinic complaining of abdominal cramping and two days later developed diarrhea with liquid and bloody stools. Endoscopic examination of the gastrointestinal tract including the colon revealed inflammation and mucosal changes. Several biopsies of the gut were taken and the patient was admitted to the hospital. Figure 3 illustrates the findings observed in one of these biopsies (low power).



*Photo courtesy of Dr. David Lewin*

Figure 3

*How do you interpret this biopsy? What would you do next?*

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### Data sheet C-4 (ctd)

#### Post-graft evolution (continued)

Low power microscopic view of a duodenal biopsy showed a decreased villous to crypt ratio, glandular loss and mucosal erosion. Brunner's glands with dilation are present beneath the *muscularis mucosa*. A submucosal diffuse mononuclear cell infiltrate, confirmed to be predominantly constituted by lymphocytoid cells on high power examination, was also apparent. The gut biopsy was compatible with a diagnosis of systemic GVHD. It was decided to prescribe additional immunosuppressive therapy. The patient was treated with intravenous methylprednisolone and improved rapidly over the next three days. Approximately 10 days later the patient was discharged from the hospital with a prescription for prednisone, which was to be tapered according to the usual protocol. One hundred days after HSCT the patient reported that he felt well. On one clinic visit at 210 days after HSCT further treatment with oral prednisolone for a skin rash was prescribed. One year after the transplant the patient was able to return to work.

*Discuss the pathogenesis of GVHD and other therapeutic alternatives not yet considered in this patient.*