

Transplantation Immunology

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BOOK D

Note to Students: The fundamental purpose of all activities in the health-care professions is to help other people. Like all behaviors, helping behavior becomes more effective and natural with practice. This workbook enables you to practice by helping your fellow students to learn basic science. Your skill at helping your fellow students should relate to your ability to help your patients in the future. This is a *Patient-Oriented Problem-Solving ("POPS")* workbook designed for four students. Before beginning this session, you should have (a) studied your class notes on this topic and the objectives designed to prepare you for it, (b) taken the pretest, and (c) reviewed the topics listed at the end of the pretest. Now, each of you should take one of the four color-coded booklets and follow the directions in it. If your group has only three students, one of you should take two booklets. Leave the remainder of the workbook intact until you are given further instructions.

Transplantation Immunology

Introduction to the Patient-Oriented Problem-Solving (POPS) System

This is a Patient-Oriented Problem-Solving activity. The purposes are

1. To help you learn how to apply your basic science knowledge to the solution of clinical problems
2. To help you learn how to better use sources (i.e., textbooks and peers) that will be available to you throughout your career

This activity consists of four phases. First, you will review the attached set of objectives, do background reading on the topics to be covered, and complete the pretest on your own. In the second phase, you will join three other students and review the pretest answers in an "open-book" discussion. In the third phase, the group will solve patient-oriented problems. Information exchange and group interaction is keys to the success of this phase. This process will allow you to teach your fellow students and, at the same time, learn from them. Finally, you will take a posttest, individually, which will enable you to assess your progress.

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Learning Objectives

1. List some of the diseases for which allogeneic hematopoietic stem cell transplantation (HSCT) or allogeneic bone marrow transplantation (ABMT) is used as a therapy.
 - A. Describe the sources of hematopoietic stem cells.
 - B. Describe how a stem cell donor is chosen.
2. Describe how the outcome (success, failure) of an HSCT is determined.
 - A. Define the goals of HSCT transplantation.
 - B. Describe how the corrective effect of an HSCT is determined.
3. Describe the immunological barriers that must be considered in doing an HSCT.
 - A. Describe host-versus-graft disease (HVGD) and graft-versus-host disease (GVHD).
 - B. Describe how an immune response can have a detrimental effect on the success of an HSCT.
 - C. Explain how the immune system of the graft recipient can be manipulated to prevent GVHD.
 - D. Explain how a graft-versus-leukemia reaction can be of benefit to the patient.
4. Describe the risks associated with HSCT.
 - A. Know the success rate of HSCT in curing a disease such as chronic myelogenous leukemia.
 - B. Describe the possibility of recurrent disease, infection, cancer, and GVHD and how these conditions may affect the success of the HSCT.
5. Describe the pathogenesis and clinical manifestations of GVHD.

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Pretest

1. Bone marrow failure is **unlikely** to be associated with:
 1. anemia
 2. increased susceptibility to infection
 3. leukopenia
 4. reticulocytosis
 5. thrombocytopenia

2. Which one of the following has **not** been used as a source of hematopoietic stem cells?
 1. Blood
 2. Bone marrow
 3. Cord blood
 4. Adult Liver
 5. Fetal Liver

3. The ideal donor of a hematopoietic stem cell transplant is:
 1. a close friend
 2. a first cousin
 3. a parent
 4. a sibling
 5. an identical twin

4. Hematopoietic stem cells are given to the recipient by:
 1. infusing the cells into the patient's bone marrow cavities
 2. infusing the cells into the patient's heart
 3. infusing the cells into the patient's blood
 4. infusing the cells into the patient's peritoneal cavity
 5. intramuscular injection

5. Patients who have myelogenous leukemia are prepared for a hematopoietic stem cell transplant by:
 1. chemotherapy and radiotherapy
 2. laparoscopic surgery
 3. splenectomy
 4. thymectomy
 5. cytokine therapy

Transplantation Immunology

Pretest

6. Which of the following is an unlikely complication of hematopoietic stem cell transplantation?
 1. Failure of the graft to survive
 2. Graft-versus-Host Disease (GVHD)
 3. Infection
 4. Malignant transformation of the transplanted stem cells
 5. Treatment-associated malignancies

7. The cells infiltrating target tissues in chronic graft-versus-host disease are:
 1. mixtures of mononuclear cells and granulocytes of donor origin
 2. mixtures of mononuclear cells of donor and recipient origin
 3. mixtures of mononuclear cells of donor origin and granulocytes of host origin
 4. mononuclear cells of donor origin
 5. mononuclear cells of host origin

8. Survival of a hematopoietic stem cell transplant and its establishment in the bone marrow may be determined by:
 1. complete blood cell counts
 2. evidence of chimerism
 3. liver biopsy
 4. lymph node biopsy
 5. measuring creatinine clearance

9. In the weeks following hematopoietic cell transplantation most patients require:
 1. folic acid megadoses
 2. interleukin-2 therapy
 3. psychotherapy
 4. red blood cell transfusions
 5. tetanus immunization

10. A risk associated with being a hematopoietic stem cell transplant **donor** is:
 1. anemia
 2. bone marrow failure
 3. infection
 4. leukemia
 5. lymphadenopathy

When you have completed the pretest, consult your study materials. Try to identify the correct answers and understand the concepts that make them correct. The list of objectives may be used as a guideline for your studies. When your group meets, you will have the responsibility of explaining some of the correct pretest answers to the others. Please bring your notes and this package to the group meeting.

Transplantation Immunology

Booklet C

Pretest Correct Answers

3. (E) is correct. One of the major barriers to successful hematopoietic stem cell transplantation is the antigens coded for by the major histocompatibility gene complex. Identical twins have identical major histocompatibility gene complexes and therefore have identical major histocompatibility complex molecules. 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. It must be noted that even when a donor is apparently identical, typing has only been performed for a relatively small number of genetic markers and there is ample room for differences in those that have not been typed. On the positive side, typing includes those markers that have a stronger influence in the outcome of a graft.

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 in recipients of hematopoietic stem cell transplants of cancer. This risk is thought to be associated with the chemotherapy and radiotherapy, which these patients receive prior to receiving the transplants. However, the malignancies originate in host tissues, not on grafted cells. Finally, not every hematopoietic stem cell transplant is successful; some of these transplants fail to thrive and localize graft in the bone marrow. Others are rejected by the host's immune system.

10. (C) is correct. The greatest risk associated with being a donor of hematopoietic stem cells is infection due to the invasion of the bone marrow by a needle in order to harvest the stem cells. Even so, infection is rare in the donors. Anemia is not encountered but in the rarest circumstance because the donors are carefully evaluated for their health and capacity of their bone marrow to produce adequate numbers of red and white blood cells for their own bodies. 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 or enlargement of the lymph gland also has not been reported.

Transplantation Immunology

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 a patient with acute 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 seven genes in the major histocompatibility complex (MHC), located on chromosome 6, which code for HLA antigens. Each of these seven genes is polymorphic meaning that there are many different versions of each gene which differ from the others by a different sequence of nucleotides. Each of these gene variants, called **alleles**, code 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, but 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 are HLA-A, HLA-B and HLA-DR, however, HLA-C and HLA-DQ may also play an important role. 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 "perfect" match is called a six-antigen match (2 HLA-A, 2 HLA-B, and 2 HLA-DR), although as previously stated there are other antigens that may be important. The HLA genes on a particular chromosome 6 constitute a "haplotype" (derived from haploid genotype). Each person has a maternal and a paternal haplotype based on which of the parent's chromosome 6s were inherited.

Transplantation Immunology

Introduction (ctd.)

The most likely place to find an 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 is to do a “family study”. 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. Each HLA antigen coded for by an MHC gene is identified by a number. 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	43	53	57
Child 2		2	6	46	11	22	15	26	40	52	55
		3	7	47	12	23	17	27	43	53	57

Transplantation Immunology

Booklet D

Pretest Correct Answers

4. (C) is correct. Technically performing a hematopoietic stem cell transplant is one of the simplest transplantation procedures. The hematopoietic stem cells are infused into a patient's blood stream using an intravenous infusion system. The surface adhesion molecules of the transfused stem cells will mediate their migration to the appropriate territory(ies) No invasive surgery or invasive procedures are involved.

7. (B) is correct. When a graft containing immunocompetent cells is placed into an immunocompromised host, the transplanted cells can recognize as non-self the host antigens. In response to these antigenic differences, the donor T lymphocytes become activated, proliferate and differentiate into helper and effector cells that attack the host cells and cause GVHD. The crucial role played by the donor T cells in GVHD is demonstrated by the fact that their elimination from a bone marrow graft avoids the reactions, although the therapeutic benefit is also significantly reduced. However, as the GVHD evolves, the majority of the cells infiltrating the different tissues affected by the GVH reaction are of host origin and include T and B lymphocytes as well as monocytes and macrophages. The proliferation of host cells is probably a result of the release of high concentrations of non-specific mitogenic and differentiation factors by activated donor T lymphocytes. Granulocytes are rarely seen in the cellular infiltrates characteristic of GVHD.

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Data sheet D

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 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⁺ stem cells are recovered. Some groups inject directly the isolated stem cells while others expand the CD34⁺ stem cells *ex vivo* with cytokine cocktails and then infuse them into the patient intravenously.

However, most groups prefer to use unfractionated CD34⁺-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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Data sheet D

Post-graft evolution (continued)

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 D

Post-graft evolution (continued)

When the patient developed fever he needed to be given 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 carbenapem (imipenem) by itself or in association with vancomycin, or an extended spectrum penicillin (e.g. piperacillin or ticarcillin) in association 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

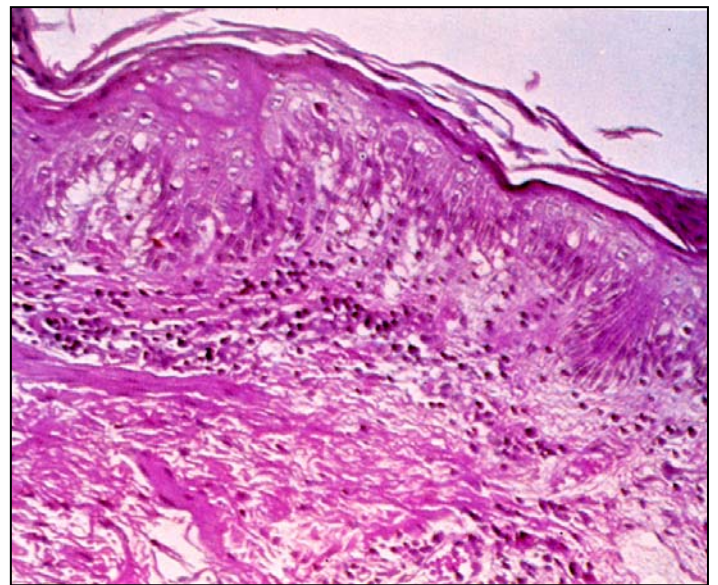


Figure 2

Photo courtesy of Dr John Maize

How do you interpret this biopsy?

Transplantation Immunology

Data sheet D

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 D

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.

Now get the concluding comments from the Web. Ask your facilitator for the password.

This will follow a post-test. Complete the post-test individually and hand it to your facilitator.