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# Organ and Bone Marrow Transplantation

M1 – Immunology Sequence

Winter 2009



# Organ and Marrow Transplantation

**Please read “Transplantation of tissues and organs”,  
pp. 338-358 and p. 123, in Parham  
before using this self-study module.**

**This presentation was adapted from lectures given  
by Dr. John Levine (Pediatrics and Communicable  
Diseases, Bone Marrow Transplantation).**

**Review of key concepts:  
Immunology of Transplantation.**

# Organ Transplantation

- Organ transplants can be performed to replace a non-functioning vital organ
- Risks include (but are not restricted to) rejection of the transplanted organ and post-transplant immunosuppression leading to infection

# ABO Matching

- ABO antigens are expressed on the vascular endothelium, hence it is important to ABO match when transplanting a vascularized organ
- ABO mismatching leads to endothelial damage by ABO antibodies with subsequent widespread thrombosis and graft loss

# **Review of ABO Reactions**

**Individual with blood group O make antibodies against type A and type B antigens, and so cannot accept grafts that are either type A, type B, or type AB.**

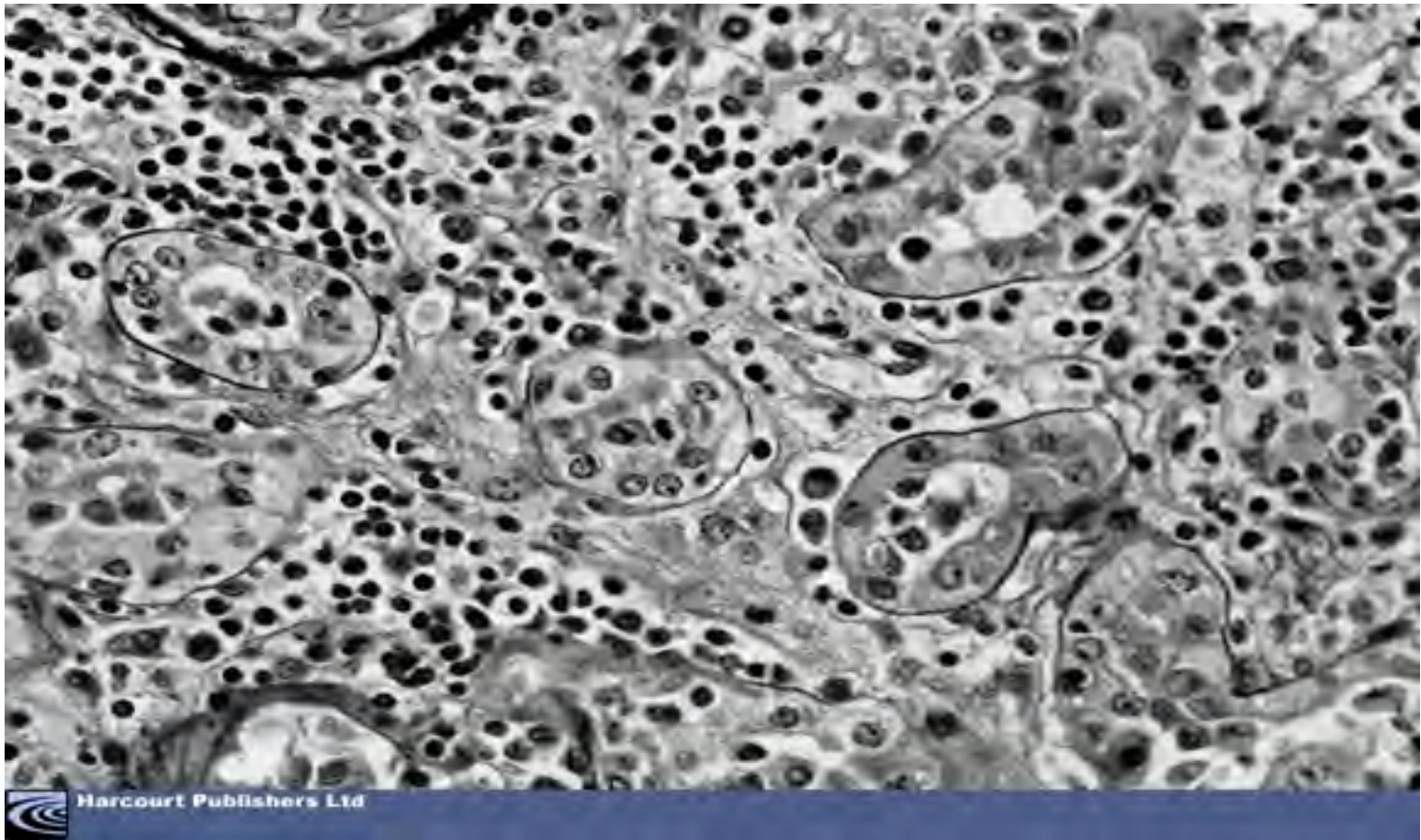
**Individuals with blood group A make antibodies against type B antigens, and so cannot accept grafts that are type B or AB. The reciprocal is true for individuals with blood group type B.**

**Individuals with blood group type AB do not make antibodies to either type A or type B antigens, and so can accept grafts from anyone.**



**Hyperacute rejection (within hours) is usually antibody mediated, most often by antibodies against A and B blood group antigens.**

**Both acute (days) and chronic (months) rejection are mediated by T cells.**



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Acute rejection. The interstitium is diffusely expanded by an inflammatory infiltrate composed predominantly of mononuclear cells (lymphocytes, monocytes).

T cells mediate graft rejection and the HLA molecules encoded in the MHC are most important in the T cell immune response to a graft.

- Syngeneic grafts (where the donor and recipient are genetically identical) will not be rejected
- If donor/recipient HLA antigens are not identical, T cells will react against foreign HLA molecules
- HLA matching reduces the chance of acute rejection
  - HLA-identical siblings are optimal
  - Post-transplant immunosuppression is needed for all transplant pairs except identical twins

# Minor Histocompatibility Antigen

- Minor histocompatibility antigens are self proteins that are loaded into the HLA peptide groove
- Even when HLA antigens are identical, differences in the self peptides derived from the donor organ can facilitate an immune response
- Thus, whenever there are genetic differences between the donor graft and the recipient T cells, an alloreaction can occur

Matching transplant donors and recipients for MHC molecules is sufficient, even though minor histocompatibility antigens are not matched.

T cell clones recognizing allogeneic MHC molecules are abundant, and thus the transplantation reaction is vigorous. It can be controlled by immunosuppression, but with difficulty. The better matched the donor and recipient are for both MHC class I and class II molecules, the fewer T cell clones that will respond and the more likely it will be the immunosuppressive drugs will be successful.

T cell clones recognizing allogeneic minor histocompatibility antigens are much less abundant, and hence the transplantation reaction is less vigorous. Immune responses to minor histocompatibility antigens are usually well-controlled by immunosuppressive drugs.

# Immunosuppression

To prevent both acute and chronic rejection, all recipients of solid organ transplants (except those between identical twins) must receive some combination of immunosuppressive drugs upon transplantation. Transplant recipients are at great risk for infections due to this immunosuppression. Even though the kinds and amounts of immunosuppressive drugs can be slowly reduced, some must be used for the entire life of the transplant recipient. Thus, these individuals are always at greater risk for infections than are healthy, immunocompetent individuals.

Immunosuppressive drugs can act in at least four different ways to suppress immune reactivity:

1. Non-steroidal anti-inflammatory drugs (NSAIDs) include aspirin, ibuprofen, acetaminophen, naproxen, and others. As discussed by Dr. Kunkel in his lecture, these reduce inflammation by inhibiting enzymes important in the synthesis of prostaglandins and leukotrienes.

2. Corticosteroids (prednisone, for example) inhibit the antigen-driven differentiation of T cells and other immune cells. Corticosteroids do this by inhibiting the expression of many different genes, some of which are important in the activation of T cells. Corticosteroids also lead to apoptosis of activated T cells. These compounds suppress the action of inflammatory cells by reducing both prostaglandin and leukotriene synthesis and by inhibiting emigration of leukocytes from blood vessels (see Dr. Stoolman's lecture). However, corticosteroids also have numerous side effects, including fluid retention, weight gain, diabetes, bone mineral loss, and thinning of the skin. These side effects are also a consequence of the ability of corticosteroids to alter gene expression.



3. Anti-metabolic drugs (azathioprine, cyclophosphamide, mycophenolate, and others) act by killing all dividing cells. Typically, these are nucleoside analogs that inhibit cell division by inhibiting nucleotide synthesis or by alkylating DNA. Thus, they act by killing T cells and B cells that are undergoing antigen-driven differentiation. Since the antigen-specific clones are eliminated as they are activated, this inhibits anti-transplant or autoimmune responses. However, all immune responses are inhibited, so the patient is also generally immunosuppressed, and thus susceptible to infection. Other side effects involve other cells with a high rate of division: bone marrow stem cells, the skin, hair follicles, epithelial cells in the intestine, the fetus.

4. The fourth group of immunosuppressive agents prevent the signaling that is important to antigen-dependent T cell and B cell differentiation. These compounds bind to cytoplasmic proteins that prevent signal transduction from the T cell or immunoglobulin receptor via calcineurin (cyclosporin A and tacrolimus) or inhibit protein translation and progress through the cell cycle (rapamycin). Cyclosporin A and tacrolimus lead to a greatly reduced expression of T cell-derived cytokines; rapamycin leads to T cell apoptosis. These agents are very effective inhibitors of antigen-driven immune responses. However, their principle side effect, general immunosuppression, is a direct consequence of this ability to inhibit immune responses.

Most commonly, two or three different immunosuppressive drugs are used to prevent transplant rejection or to improve rheumatologic diseases or allergic diseases. By using smaller amounts of each drug, and by using drugs from more than one of the four groups of immunosuppressive agents, inhibition of immune responses can be improved, while avoiding some of the side effects on other organ systems. However, general immunosuppression cannot be avoided, and so patients on immunosuppressive drugs are always at risk for opportunistic infections by bacteria, viruses, and fungi.

There are three ways to match donors and recipients for histocompatibility antigens.

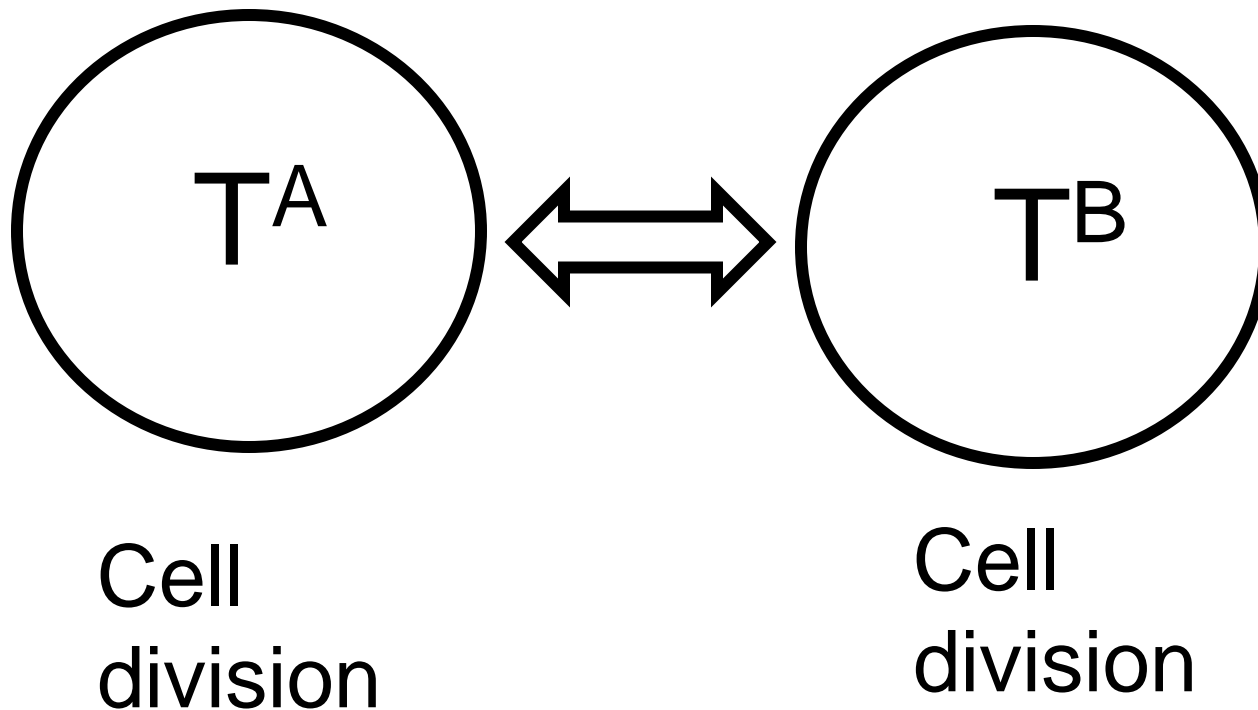
1. Type for MHC molecules expressed on leukocytes using antibodies. This is the original method used. Antibodies specific for a single allele of one MHC gene are difficult to obtain, and many alleles (especially those encoded by class II genes) can not be typed with antibodies.
2. Type for MHC genes by PCR amplification and sequencing. This has the advantage of finding all potential differences at the MHC, but is expensive and takes more time than typing with antibodies. If time is not a factor, this is the method of choice.

A third way to test for a match of transplantation antigens is a “Mixed Lymphocyte Reaction” (MLR-- review Parham, pg. 123).

An MLR is a co-culture of lymphocytes from two different individuals

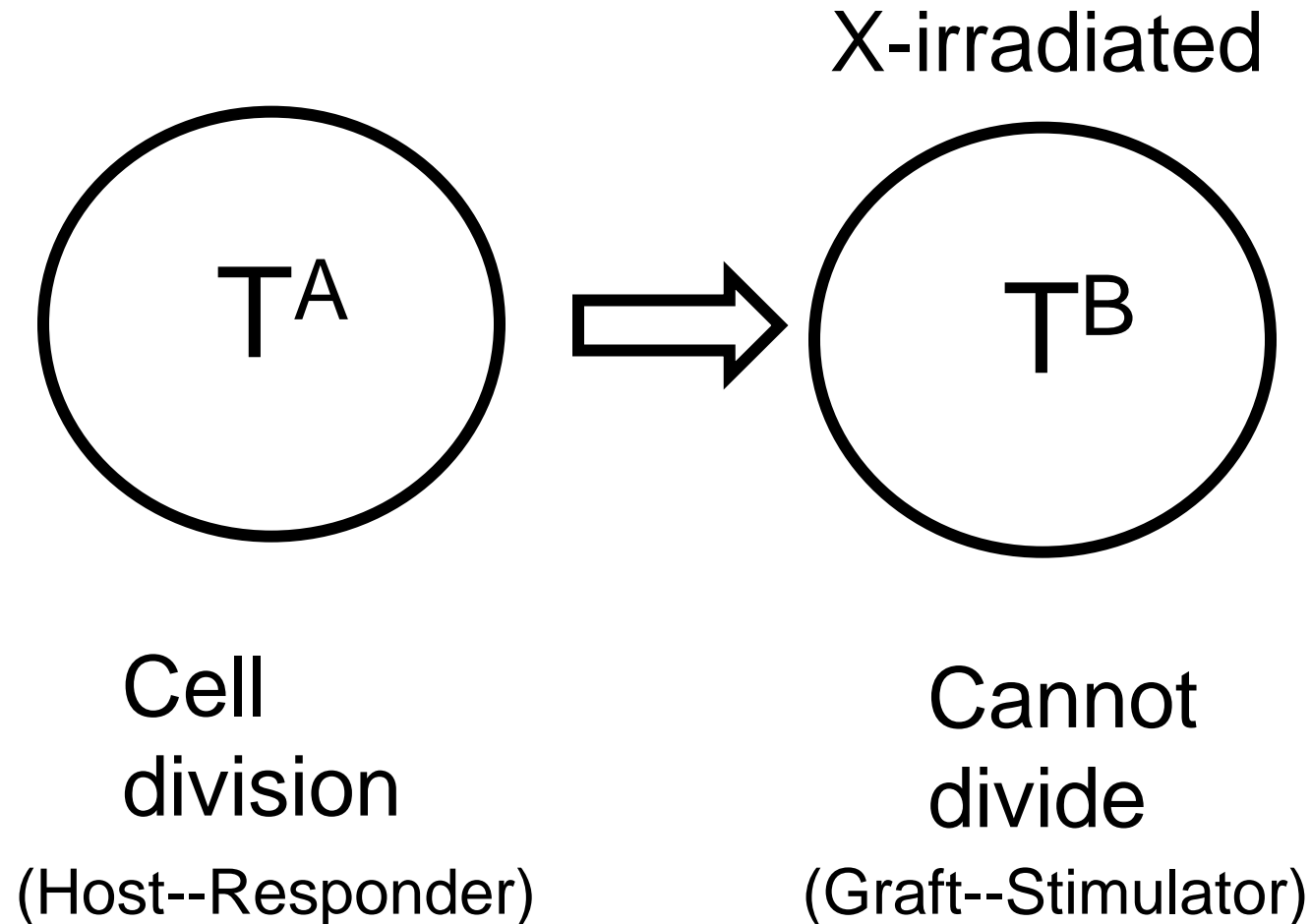
The MLR detects the ability of T lymphocytes to recognize allogeneic differences on white blood cells from another individual. After recognizing the differences, the T cells divide. This cell division can be measured by uptake of tritiated thymidine from the culture media into the cells.

Lymphocytes from individual “A” mixed with lymphocytes from individual “B”: T cells from each individual recognize transplantation antigens on the other individuals lymphocytes as foreign, and begin to undergo cell division in the activation phase of the immune response.



**A negative control for the MLR is the potential graft recipients lymphocytes reacting to his or her own x-irradiated lymphocytes. Since there is no “foreignness” in this combination, the amount of cell division measured by uptake of a DNA precursor, tritiated thymidine, is the background level. The test combination is the recipient’s lymphocytes (“Responders”) reacting to a potential donor’s x-irradiated lymphocytes (“Stimulators”). Since the donor’s lymphocytes are x-irradiated, they cannot divide. Hence, one measures the cell division resulting from a “one-way” recognition of the donor (graft) by the recipient (host).**

# MLR that measures “One-way recognition”





# Mixed Lymphocyte Reaction (MLR)

Almost all of the cell division is the result of recognition of MHC-encoded differences. Most importantly, differences at class II, which are covered poorly by tissue typing tests, are the key differences that result in thymidine uptake.

Thus, tritiated thymidine uptake in MLR is a very sensitive measure of immunological recognition between a potential donor and graft recipient. Since the same T lymphocytes that might eventually lead to graft rejection are the reagents used, availability of suitable reagents is not an issue.

The mixed lymphocyte reaction takes about one week to complete, and it is expensive. It has the advantage of measuring the histocompatibility differences by a T cell mediated immune response. Thus, it estimates well the intensity of a transplantation reaction in vivo without using any specific reagents (antibodies).

# Bone Marrow Transplantation

- Bone marrow transplantation can be thought of as another type of organ transplant
- In the case of marrow transplantation, alloreactivity is not only in the direction of host versus donor graft, ie, rejection
- Alloreactivity also takes place in the direction of donor versus host, ie, graft-versus-host disease

# Hematopoietic Stem Cell Transplantation

Hematopoietic stem cells are effective after transplantation because the stem cells home to the bone marrow from the peripheral blood and a few stem cells can reconstitute all of the components of the bone marrow.

Graft versus host disease (GVHD) is caused by mature T cells that contaminate the bone marrow or stem cell preparation. These donor T cells recognize the allogeneic MHC molecules of the host, or the minor histocompatibility antigens of the host, and initiate an inflammatory immune response against host tissue.

# Donor Selection Influences Risk of GVHD

In order of increasing differences at HLA, and therefore decreasing desirability for a bone marrow donor:

- Syngeneic
- Genotypic-identical sibling
- Other family member
- Matched unrelated donor
  - The probability that any two unrelated persons will match is extremely low
  - Broad application of this technique is made feasible by large volunteer registries

# Conditioning Regimens

Recipients of bone marrow transplants invariably undergo a conditioning regimen with chemotherapy and/or radiation in order to:

- Reduce the tumor burden with cytotoxic drugs
- To immunosuppress the recipient to prevent rejection of the bone marrow transplant

# Transplant Complications

- Toxicity from the conditioning regimen
  - Short and long term effects
- Graft rejection
- Graft versus Host Disease



# Acute GVHD

- Acute GVHD develops usually develops within the first 100 days from the transplant
- Primary targets are skin, liver, and intestines

# Acute GVHD of Skin



# Acute GVHD of Skin



# Chronic GVHD

- Chronic GVHD which clinically resembles an autoimmune disease generally does not develop prior to day 100
- Target organs include the skin (scleroderma), dry eyes, mouth and vagina (sicca like syndrome), liver, GI tract (diarrhea, anorexia, nausea), lungs (bronchiolitis obliterans), fasciitis, serositis.

Please answer the following questions to estimate your mastery of the material on transplantation.

Which scenario is MOST LIKELY to result in rejection of a solid organ transplant?

- A. Cadaveric kidney transplant from blood type A DONOR into blood type AB RECIPIENT.
- B. Cadaveric kidney transplant from blood type O DONOR into blood type AB RECIPIENT.
- C. Cadaveric kidney transplant from blood type AB DONOR into blood type O RECIPIENT.
- D. Syngeneic kidney transplant from one sibling into another.
- E. Cadaveric kidney transplant from blood type O DONOR into blood type O RECIPIENT.

The correct answer is “C”. Naturally occurring antibodies to type A and type B blood group are made by the Type O recipient. These antibodies would react with the vascular endothelium of the graft, leading to hyperacute rejection.

Rejection of grafts by the host, and graft versus host disease:

- A. Are both caused by T cell recognition of allogeneic differences.
- B. Are both caused by T cell recognition of bacterial infections.
- C. Are both caused by antibodies binding to ABO antigens.
- D. Are both Type I reactions.
- E. Are both characterized by a lack of inflammation.



The correct answer is “A”. None of the other answers are correct. ABO incompatibility can lead to graft rejection, but is not a cause of graft versus host disease.

Which is the most important in matching transplant donors and recipients?

- A. Sex
- B. Age
- C. Size
- D. Immunoglobulin allotype
- E. MHC alleles
- F. Minor histocompatibility alleles
- G. Complement

The correct answer is “E”. MHC matching is the most important factor. Size is a factor, because the new organ must fit into the space available. ABO matching is very important, but was not offered as an answer. Minor histocompatibility antigens are relatively less important. Finally, there are so many different minor histocompatibility genes, that it would be impossible to type even a portion of them.

Recipients of solid organ transplants are at risk for infection because:

- A. They had a malfunctioning organ for a long time.
- B. They received a blood transfusion during surgery.
- C. They cannot use antibiotics.
- D. They have been immunosuppressed to prevent graft rejection.
- E. They cannot produce antibodies.

The correct answer is “D”. Immunosuppression leads to poor T cell and B cell activation following recognition of a pathogen, which in turn leads to a poor immune response against that pathogen.

Acute graft-versus-host disease (GVHD) is caused primarily by:

- A. The patient's T cells that become activated by recognizing donor's APC.
- B. The patient's APC that become activated by donor's T helper cells.
- C. The donor's T cells that become activated by recognizing the patient's APC.
- D. The donor's APC that become activated by the patient's T helper cells.

The correct answer is “C”. T cells contaminating the donor bone marrow recognize allogeneic class I and class II MHC molecules on the recipients antigen presenting cells, and initiate an immune response that is manifest as graft versus host disease.

MLR (mixed lymphocytes reaction) results from a potential kidney recipient and his family members. Results are given as cpm of tritiated thymidine incorporated X10<sup>-3</sup>. Hence, 1=1000, 6=6000, 2=2000, 20=20,000, etc.

Stimulator cells

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Responder

Cells	Patient	Mother	Sibling 1	Sibling 2	Unrelated
Patient	1	11	1	31	20
Mother	6	1	45	7	14
Sibling 1	1	39	1	22	27
Sibling 2	20	5	26	1	15
Unrelated	30	16	39	20	1

Which is the MOST LIKELY result of tissue typing for HLA A, B, C, and DR in this family?

- A. The patient and the mother will be HLA identical.
- B. The patient and Sibling 1 will be HLA identical.
- C. The patient and Sibling 2 will be HLA identical.
- D. The patient and Sibling 1 will differ by one HLA haplotype.
- E. The patient and the unrelated individual will be HLA identical.

Who would be the best kidney donor to the patient?



The correct answer is “B”. Since the patient and sibling 1 do not stimulate each others T cells to divide in tissue culture (in both directions), they must be identical at the MHC, and must be very similar at many minor histocompatibility genes.

Sibling 1 would be an ideal donor for a kidney or a bone marrow transplant. Since the patient does not recognize his or her cells as foreign, the patient would not reject the graft. Since the T cells from sibling 1 do not recognize the patient as foreign, graft versus host disease in a bone marrow transplant is less likely also.

A 32 year old woman who received an allogeneic bone marrow transplant from her sister 25 days ago complains of watery diarrhea. On physical exam she has developed a diffuse erythematous maculopapular skin rash. Her blood counts reveal normal WBC, mild anemia and moderate thrombocytopenia as expected at this point in time following her BMT. Based on your knowledge of BMT, which course of action seems MOST appropriate:

- A. Administer an infusion of donor T cells to prevent impending graft rejection.
- B. Begin steroid therapy for probable acute graft-versus-host disease.
- C. Initiate broad spectrum antibiotic therapy for likely skin infection.
- D. Both A and C.
- E. ALL of the above.

The correct answer is “B”. The diarrhea, the skin rash, and the timing (25 days post bone marrow transplant) indicate graft versus host disease. Recall that the skin and the gut are two of the three target organs for acute graft versus host disease.

Immunosuppression, while avoiding some of the side effects on other organ systems, is achieved by:

- A. Stopping immunosuppressive drugs a few days after transplantation.
- B. Using very high doses of a single immunosuppressive drug for a short time.
- C. Using a combination of two drugs from the same group of immunosuppressive drugs.
- D. Using smaller amounts of two or more immunosuppressive drugs, each with a different mechanism of action.
- E. Immunosuppression without dramatic side effects on other organ systems cannot be achieved.

The correct answer is “D”. If you gave another answer, you should review slides 11-16.

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