Chapter 18 Emergent Properties at the Organismal Level

A grove of quaking aspen, *Populus tremuloides*, in Richfield Ranger District, Fishlake National Forest, Utah, United States. Many of these trees may be part of just one individual organism, with connected roots.

Learning Objectives

1. Review how the mammalian immune system distinguishes self vs. non-self.
2. Describe what an individual organism is, comparing your preconception of the individual to your conception of it after studying section 18.2.
3. Explain how an individual is an emergent property, and how an individual can sometimes seem like a population.
4. Explain how emotions arise and how they illustrate the big idea of emergent properties at the level of the individual organism.
5. Describe observational methods and experimental techniques for studying emotions.
6. Restate the disposable soma theory of aging and illustrate your understanding by citing eukaryotic and prokaryotic examples.

Bio-Math Exploration Learning Objectives

Ethical, Legal and Social Implications Learning Objectives

1. Evaluate the pros and cons of using prescription drugs to modify, or normalize, behavior in children with ADHD.
2. Consider the implications of altering the timing of death.

Chapter 18 Outline

Introduction

18.1 How can a mother tolerate her fetus?

18.2 What is an individual?

18.3 What is the source of emotions?

Ethical, Legal and Social Implications Box 18.1: Should prescription drugs be used to normalize behavior in children?

18.4 Why do individuals age and die?

Ethical, Legal and Social Implications Box 18.2: Should we alter the timing of death?

Conclusions

You Are Here		Big Ideas of Biology				
		Information	**Evolution**	**Cells**	**Emergent Properties**	**Homeostasis**
Levels of the Biological Hierarchy	**Molecules**	**Chapter 1**	**6**	**11**	**16**	**21**
	Cells	**2**	**7**	**12**	**17**	**22**
	Organisms	**3**	**8**	**13**	**18**	**23**
	Populations	**4**	**9**	**14**	**19**	**24**
	Ecological Systems	**5**	**10**	**15**	**20**	**25**

Vignette here

18.1 How can a mother tolerate her fetus?

- Context: Two unrelated individuals cannot exchange organs and yet a mammalian mother is able to carry a fetus that is 50% unrelated to her.
- Major Themes: Randomness within a biological system provides flexibility of response; and biological systems require resources, which results in competition or cooperation.
- Bottom Line: The mammalian immune system rejects non-self tissue but pregnancy provides immune protection to the non-self fetus.

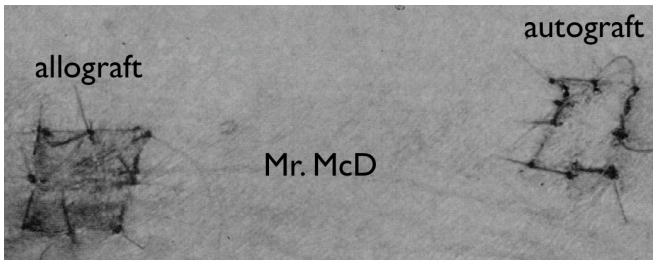
You are familiar with two facts from your everyday life and yet when you consider them carefully, they contradict each other. The first fact is that a pregnant woman carries a fetus inside her body for nine months to nurture and protect it from harm. The second fact is that successful organ transplantation requires the donor and the recipient to be matched so they are compatible or else the donated organ will be rejected by the recipient's immune system. The contradiction is that the father of a fetus is unrelated to the mother, requires no tissue matching, and yet the mother's immune system tolerates the fetal tissue that is 50% foreign. How can a pregnant woman tolerate a fetus for nine months? The answer to this question is an emergent property at the individual level and the focus of Section 18.1.

To understand how mothers can tolerate their fetuses, you have to start at a very unlikely place – a British military field hospital during World War II. In particular, you will follow the observations of Peter Medawar which ultimately led to his winning a Nobel Prize in Physiology or Medicine in 1960. One of the terrible consequences of war are injuries that need surgical intervention, and Medawar treated many soldiers whose skin was badly burned. Burned skin needs to be replaced by healthy skin, a **graft**, which must be transplanted, or grafted, to the burned area. If the patient has only small areas of skin burned, the replacement skin graft can be taken from another part of the patient's body. Tissue transferred from one part of an individual's body to another part of the same person is called an **autograft**. Autografts are the most successful because the transplanted skin cells are genetically identical to all the other cells in the patient's body. However, when a patient is burned over a large portion of his or her body, there is not enough healthy skin to graft onto all the burns and another source of healthy donor skin is needed. **Allografts** are taken from another person and transplanted to the patient in hopes the skin graft will grow and fill in the damaged areas. Many surgeons before Medawar had discovered that autografts were very successful but allografts often turned black and died. Medawar's big insight was recognizing a pattern and deducing a mechanism to explain why allografts were rarely successful. {*Definitions*: **Grafts** are pieces of skin surgically inserted into a different location. **Autografts** are transplanted tissue from one place to another on the same individual. **Allografts** are transplanted tissue from one individual to another.}

Like any good scientist, Medawar's observations stimulated him to ask some questions which he could answer through careful experimentation (Figure 18.1). Medawar took small patches of skin from healthy volunteers and transplanted them to different locations. In Figure 18.1a, you can see the outcome for a "Mr. McD" which is similar to the types of graft Medawar performed. Notice how small the skin patches are by comparing the size of the stitches to the overall graft size. When an allograft was rejected by the host recipient, Medawar called this **first-set rejection**. In some cases, Medawar transplanted a second skin graft from the same

donor onto the same recipient and if it was also rejected by the recipient, Medawar called this **second-set rejection**. After World War II, Medawar published a series of important papers describing a wide range of similar skin graft experiments he performed using rabbits instead of humans (Figure 18.1b). Although you cannot see it in Figure 18.1a, Medawar described in detail the stages of allograft rejection which included redness and swelling of the area prior to the tissue turning black and dying. In some experiments, he compared the rejection rates depending on whether the second allograft was surgically placed at the same site as the first one, or somewhere else. {*Definitions*: **First-set rejection** happens when an allograft is transplanted onto a recipient for the first time. **Second-set rejections** happen after the same donor tissue is transplanted onto the same recipient as the first-set rejection.}

a)



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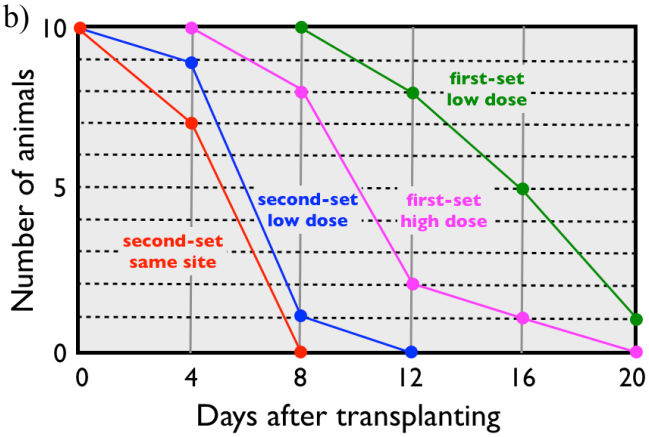


Figure 18.1 Discovery of autograft acceptance and allograft rejection. (a) Volunteer "Mr. McD" had two skin patches sewn onto his arm. The allograft came from an unrelated man and the autograft came from Mr. McD. (b) Averaged number of rabbits with skin grafts intact from five replicates of ten rabbits each, showing the time course of allograft rejection.

Integrating Questions

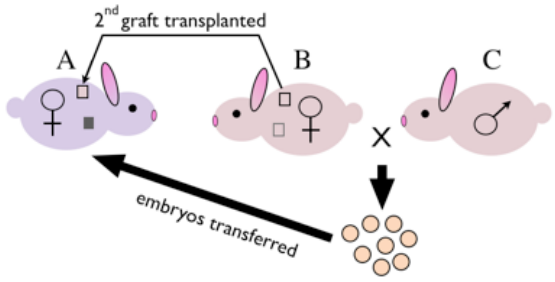
1. Explain the logical contradiction between a pregnant woman carrying a fetus and allograft rejection. Given that tissue near allograft transplants swell and turn red prior to dying, what physiological process do you think causes the rejection of the foreign tissue?
2. Analyze the rabbit data in Figure 18.1b and describe differences between first-set and second-set rejections. What is the consequence of a large (high dose) allograft vs. a small (low dose) allograft? Although the data lack error bars, do you think there is a significant difference between second-set rejection at the same site vs. a second-set rejection at a different site (low dose)?

Medawar performed hundreds of skin graft experiments to quantify the rate of rejection and found that large skin patches are rejected faster than small patches as long as both were first-set rejections. Second-set rejections occur faster and the time course of the rejection is not significantly influenced by whether the graft is transplanted to the same site or a different site. Based on the redness and swelling, Medawar correctly hypothesized that allograft rejection is caused by an immune response. Autografts are "self" tissue meaning the tissue is genetically identical to the recipient while allografts are "non-self" tissues and rejected the same way infections are rejected by inflammation. Given that immune systems attack and reject non-self tissue, the contradiction of a pregnant mammal is more apparent. By definition, a fetus is 50% non-self and therefore should be rejected as an allograft, but you know fetuses are not rejected. This contradiction stimulated a very compelling set of experiments to understand why pregnant

women fail to reject the non-self fetal tissue inside them.

By the 1960s, biologists accepted that the immune system rejected allografts but not fetal tissue. Two possible explanations were proposed: 1) pregnant women have a reduced immune function and thus a fetus slips past a weakened immune system or 2) fetal tissue benefits from a protected status through some unknown mechanism. A group of immunologists tested these two hypotheses directly in a beautifully designed experiment (Figure 18.2). Female rabbit A receives an allograft from female rabbit B and the allograft is rejected (dark square on rabbit A) as expected. After the allograft is first-set rejected, rabbit B is mated with rabbit C and the fertilized embryos are transferred to the uterus of rabbit A. Rabbit A receives a second allograft from rabbit B either at the time of the embryo transferral, or halfway through the pregnancy of rabbit A. The experiment in Figure 18.2 is technically challenging and not every transferral of embryos was successful, but a general trend was evident (Figure 18.2b and 18.2c).

a)



b)

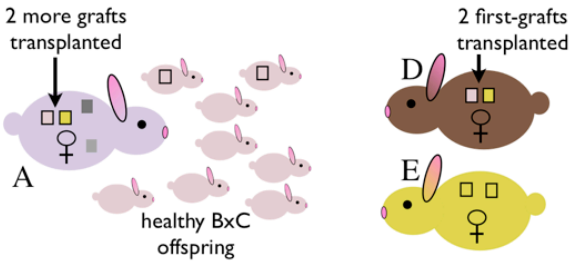
c)

results	2nd graft transplanted	
	at transferral	mid-pregnancy
babies	3	7
no babies	1	2

Figure 18.2 Allografts rejected but embryos not rejected. (a) Foster mother A was implanted with embryos from the mating of rabbits B and C. Rabbit A had already rejected one allograft from rabbit B prior to embryo transfer and second allograft transplantation. (b) Results from embryo transfer. (c) The number of pregnancies that produced offspring and the timing of the second allograft transplantation.

After analyzing this first set of data, the investigators designed a more rigorous experiment to directly test the ability of rabbit A to reject non-self tissue (Figure 18.3). After giving birth to a healthy litter, rabbit A received two more allografts. One graft was from one of her foster offspring, and the other allograft was from a new, unrelated rabbit E. As a control, the immunologists also transplanted equivalent allografts from rabbit A's foster offspring as well as unrelated rabbit E onto a third, unrelated rabbit D. All of the rabbits in this experiment were female and the data are summarized in Figure 18.3b.

a)



b)

experimental conditions	baby skin transplanted to:		rabbit E skin transplanted to:	
	foster mother A	unrelated rabbit D	foster mother A	unrelated rabbit D
average days graft survived	4.0 *	6.5	6.0 *	7.0

* indicates $p < 0.01$; experiment replicated 5 times

Figure 18.3 Follow up experiment from Figure 18.2. (a) After raising her offspring, mother A receives two more skin grafts, one from her BxC offspring and one from unrelated female E. Unrelated rabbit D receives two equivalent allografts. (b) Quantified results showing the average number of days skin grafts survived on recipient rabbits.

Integrating Questions

3. Does rabbit A retain her immune function while pregnant? Does rabbit A's immune system prevent her from carrying fetuses that are 100% non-self? What percentage of the foster mothers were able to deliver healthy offspring? Support your answer with data from Figure 18.2.
4. Did rabbits A and D in Figure 18.3 reject the skin grafts from rabbit E at the same rate? Were the skin grafts from the offspring rejected at the same rate by both rabbits A and D? Explain any significant differences you see in the rejection rates for rabbits A and D.

The experiment in Figure 18.2 directly tests whether pregnant females have weakened immune systems. If pregnant females had reduced immune function, you would not expect them to reject allografts, but they do reject allografts regardless of when the second-set skin transplants were given to the recipient rabbit A. About 75% of the foster mothers are able to carry the implanted embryos to term and deliver healthy offspring even though these experimental offspring are 100% non-self instead of 50% non-self. The immune systems of both rabbits A and D rejected the allografts from the BxC offspring as well as the new rabbit E. Rabbit D rejected both allografts at about the same rate of 6.5 and 7 days, but rabbit A had a different response. Rabbit A rejected its foster offspring significantly faster ($p < 0.01$) than it rejected the allograft from rabbit E even though both patches of skin were transplanted at the same time. The offspring allografts were rejected faster because they were second-set transplants while the allograft from rabbit E was a first-set rejection. The experiment also demonstrated that immune rejections of allografts are specific and do not occur at equal rates. In particular, rabbit A rejected allograft from her BxC foster offspring because rabbit A had previously been exposed to skin from rabbit B. Rabbit A rejected allograft E slowly because rabbit A had not been exposed to tissue from rabbit E before. Immune responses are specific for the source of non-self tissue, and immunity does not uniformly affect all allografts equally.

At this point in the Section, you can see the progress that was made between Medawar's initial observations and the experiments in the 1960s with the pregnant rabbits. The fetus must posses some mechanism to protect itself from the mother's completely functional immune system. However, the immune system is a difficult area of physiology to study and many big questions remained unanswered about how the normal immune system works. How does the immune system recognize one allograft for second-set rejection quickly while at the same time more slowly reject a first-set allograft? What cells are responsible for "remembering" a previous exposure to a particular source of non-self tissue? Although rabbits were the model system of choice early on, many current immunologists prefer to work with mice, in part because they are smaller, less expensive to maintain, reproduce faster and investigators have many more mutant strains of mice to help dissect the role of particular genes.

As with rabbits, mice respond differently to first-set and second-set allografts (Figure 18.4). Second-set rejections happen faster than first-set rejections in mice as in rabbit and humans. In this experiment, the investigators isolated the white blood cells of the immune system and separated them into different sub-types such as B cells, T cells, and natural killer cells. The immunologists injected **naïve** mice with different sub-types of white blood cells from a mouse that had already exhibited first-set rejection. Shortly after injecting a naïve mouse, the investigators transplanted allografts that had been rejected previously by the white blood cell donor. {Definition: A immunologically **naïve** individual is one that has not been presented with non-self tissue.}

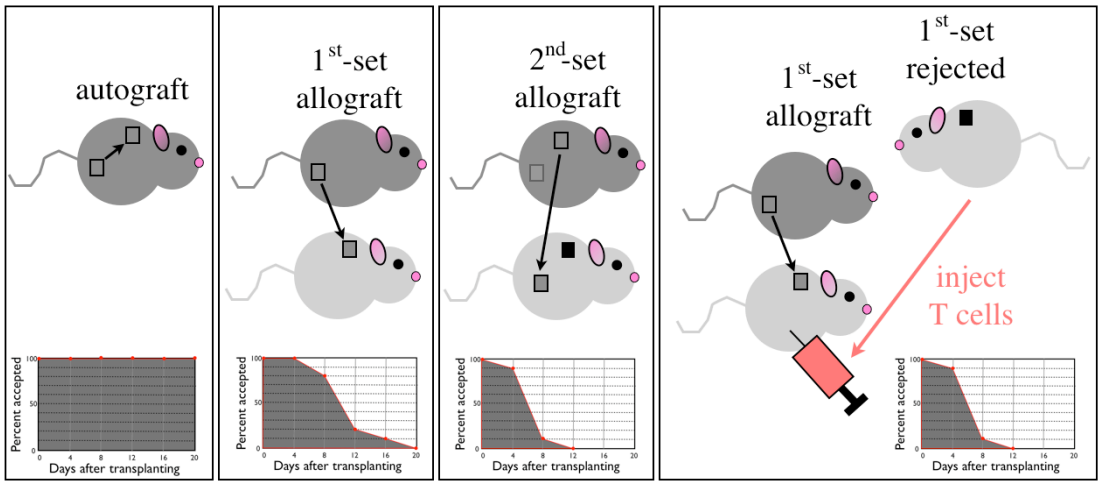


Figure 18.4 Rate of graft rejection affected by the state of recipient mouse. Mice were given grafts as diagramed and the outcome of the grafts are plotted as percent of grafts accepted (shaded areas) over 20 days. The injected mouse was immunologically naïve prior to the injection and first-set transplant.

Integrating Questions

5. How do second-set rejections in mice compare to those in rabbits and humans?
6. When a naïve mouse is given a first-set allograft, how many days does it take for the recipient to completely reject the graft? How many days does it take for a second-set rejection to reach completion? What sub-type of immune cells are responsible for the "memory" of previous allograft exposure?

Second set rejection is caused by T cells, a sub-type of white blood cells. In mice, first-set rejection takes about 20 days while second-set rejection only takes about 12 days. This experiment was a critical breakthrough in understanding the normal immune system. Immunologists would need to understand a normal immune system before they could determine how a fetus avoids being rejected. Another critical component of the immune system was the discovery shown in Figure 18.5. With a few minor exceptions, every cell in a mammal's body displays many copies of the **major histocompatability complex type one** (**MHC I**) molecule. MHC I molecules are integral membrane proteins as illustrated by the thin line passing from the outside of the cell into the cell's cytoplasm, but the vast majority of MHC I protrudes into the extracellular world. MHC I proteins look like moose heads and between their antlers is an empty space. However, the thousands of MHC I molecules found on the surface of each cell never have empty spaces – the space is always occupied by a protein fragment, or **peptide**, that was made inside the cell displaying the MHC I molecule. MHC I plus peptide is a cell's way of defining "self". In other words, MHC I molecules display fragments of every protein made inside that cell like a proud grandparent showing photos of its grandchildren. All the cells of an organism define "self" by displaying peptide fragments from every protein produced inside all the cells of an individual. {*Definitions*: The **major histocompatability complex** is a region of the genome that encodes the **type one** proteins (MHC I). **Peptides** are smaller protein pieces derived from a larger protein.}

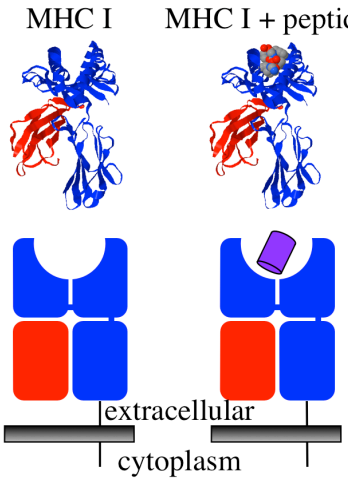


Figure 18.5 Structure of class I major histocompatibility complex (MHC I) protein. MHC I protein is composed of two subunits and contains a binding groove for protein fragments made inside? the cell

displaying the fragment. Three dimensional structures are on top, stylized diagrams below; purple barrel represents a self peptide.

The discovery of MHC I molecules was another major milestone in understanding how the immune system works. The rejection of allografts and acceptance of autografts made sense with the recognition of MHC I presenting self-peptides on the surface of skin graft cells (Figure 18.6). Autograft cells display on their surfaces MHC I molecules and inform the T cells that the source of the skin graft is self and the cells are never rejected. Allografts display peptides from proteins made inside non-self cells and thus are recognized as foreign by the recipient's T cells. However, the experimental data in Figure 18.6a uncovered an important characteristic of the MHC I molecules. Through careful breeding of mice, immunologists produced different strains of mice that were identical at every locus except MHC I. Conversely, immunologists bred mice that were identical at the MHC I locus but varied at other loci. Therefore, immunologists could distinguish the impact of MHC I differences vs. the impact of the peptide differences on allograft rejection (Figure 18.6b).

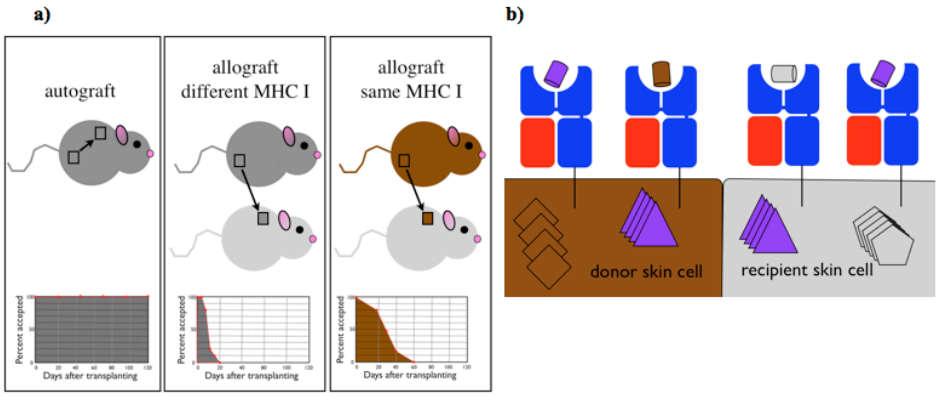


Figure 18.6 Cells display self-peptides in MHC I. (a) Mice were given grafts as diagramed and the outcome of the grafts are plotted as percent of grafts accepted (shaded areas) over 120 days. (b) Adjacent skin cells from a graft and recipient mouse cell display fragments of proteins made in their cytoplasms with identical MHC I proteins.

Integrating Questions

7. Look at the structural image of MHC I with a peptide in Figure 18.5. If you were a T cell trying to distinguish self from non-self by feeling this structure, which part or parts do you think you would feel to inform you? Since MHC I molecules only display peptides made within the cell on which they are presented, do you expect the peptide to bind weakly or tightly to the MHC I molecule displaying it?
8. How many days does it take a recipient mouse to first-set reject an allograft when the MHC I molecules are different between the donor and the recipient? How many days does it take a recipient mouse to reject an allograft when the MHC I molecules are identical between donor and recipient? Which molecule plays the bigger role in determining the rate of rejection – the peptide or MHC I? Support your answer with data.
9. Does a T cell interact, or feel, only the MHC I molecules, only the peptide fragments, or both? Support your answer with data from Figure 18.6. Go to this online tutorial (see http://www.bio.davidson.edu/courses/immunology/chime/mhc/2FRMCONT.HTM as an example) that allows you to better understand the relationship between an MHC I molecule and its bound peptide.

Since MHC I molecules display self protein fragments, these peptides must bind tightly to prevent other peptides from binding to an empty space and incorrectly appearing as a self

peptide. T cells use their receptors to "feel" the surfaces of all cells to distinguish self from non-self. In their efforts to recognize cells, T cell receptors physically interact with the peptide and MHC I molecules as you might imagine when you study their structure in Figure 18.7a. The peptide and the "antlers" of MHC I molecules are about the same height which allows a T cell receptor equal access to both. Unlike autografts which are never rejected, allografts containing different alleles of MHC I are first-set rejected in 20 days in mice. However, the immune system of mice take 60 days to first-set reject allografts with identical MHC I alleles as the recipient but displaying non-self peptides as defined by the recipient's T cells (see Figure 18.6b). Therefore, T cell receptors utilize MHC I as the primary means for rejecting non-self tissue which is how the name *major* histocompatibility was chosen for the molecules encoded by the MHC locus. Tissue with different MHC I alleles are not compatible for transplantation and are rejected as non-self. In addition, T cells can also initiate rejection if the peptides are perceived as non-self even if the MHC I proteins appear to be self. Distinguishing non-self peptides within self MHC I is how viruses are detected and destroyed by T cells when your cells become infected. These data highlight the fact that T cells must be "educated" to distinguish self peptides from non-self peptides.

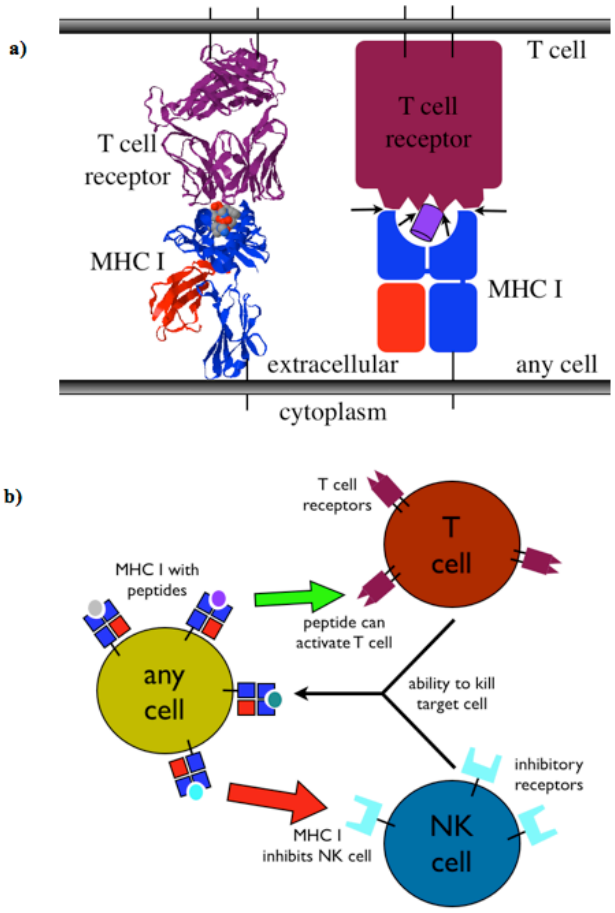


Figure 18.7 Immune cells kill non-self cells. (a) T cells recognize self-cells through their receptors that touch both MHC I molecules and their presented peptides (arrows). Three dimensional structure is on left; diagram on the right. (b) All cells use MHC I + peptide to identify themselves to T cells and Natural Killer (NK) cells.

T cells are essential to the vertebrate immune system (Figure 18.7b). T cells interact with every cell in your body and use their receptors to determine the identity of each cell as self or non-self. If the MHC I molecule is "familiar", the T cell perceives the cell as self. If the peptide bound to MHC I is "familiar", then the T cell recognizes the self cell as containing self proteins and the T cell moves on. However, if the T cell does not recognize the MHC I protein or does not recognize the peptide as self with a self MHC I, then the T cell has the capacity to kill the offending cell. Virally infected cells display viral proteins within MHC I molecules and thus recruit T cells to kill the infected cells and all the viruses within the infected cell. Some viruses try to avoid being displayed by their host cells and block the movement of MHC I molecules to the surface of the infected cell. In these cases, a different sub-type of white blood cells called **natural killer cells** will destroy any cell that lacks MHC I on its surface. MHC I molecules bind to inhibitory receptors on natural killer cells to prevent the natural killer cells from attacking all self cells. Therefore, every cell must display MHC I plus peptide to avoid being attacked by natural killer cells. If the cell displays non-self peptides, the cell will be killed by T cells. T cells

and natural killer cells of a pregnant mother maintain their immune function but somehow these two white blood cells fail to kill fetal cells which are, by definition, non-self. {*Definition*: **Natural killer cells** destroy any cell that lacks MHC I molecules on its surface.}

By now you should be convinced that the immune system normally rejects all allograft cells as non-self either because they contain different MHC I alleles, because the peptides are non-self, or both. Immunologists continue to research how the immune system works as many aspects remain unknown. However, contemporaries of Medawar wanted to know if the immune system could distinguish male from female cells (Table 18.1). The investigators had bred mice carefully so that all offspring carried identical alleles on every chromosome so that the only differences were the 53 genes unique to the Y chromosome present only in males (see a summary here http://www.ncbi.nlm.nih.gov/projects/mapview/maps.cgi?taxid=10090&chr=Y#summary). The immunologists performed a series of allografts with these highly inbred mice and determined the rate of graft rejection. In one set of allografts, they first injected the female recipients with mouse sperm cells and transplanted the skin graft 14 days after the injection to determine the rate of allograft rejection with or without previous exposure to male cells.

Table 18.1 Gender effects on graft rejection rates for genetically identical, inbred mice.

donor → recipient	number of animals	percent rejected	average days to rejection ± stdev
male → male	16	0	NA
female → female	15	0	NA
female → male	15	0	NA
male → female	15	100	28 ± 3
male → primed female ^	10	100	14 ± 2

^ Primed female injected with sperm 2 weeks prior to skin graft.
Modified from Katsh *et al.*, 1946; their Table 1.

Integrating Questions

10. Go to the interactive Jmol tutorial need tutorial with buttons using PDB ID# 1OGA similar to Firstglance version) and determine how many places this T cell receptor touches the MHC I molecule and how many places the receptor touches the peptide. Which molecule physically interacts with the T cell receptor more?
11. Search the internet (WileyPlus?) for the term "nude mouse" to see how it got its name. What is the immune system phenotype for this mutation? Given the phenotype, do you think this mouse could live a healthy life in a normal environment? Explain your answer. Search OMIM using the identifying code 242700 (see http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=242700). What is the consequence of this human mutation and how does it compare with the immune system phenotype of the nude mouse?
12. Given the data in Table 18.1, can male mice accept female skin? Explain why given what you have learned so far. Does prior exposure to male cells influence graft rejection in male → female allografts? Explain why based on what you have learned so far. What might you expect a pregnant human mother to do to every male fetus given the data in Table 18.1?
13. Hypothesize how the fetus avoids destruction by T cells and natural killer cells. Given that skin allografts are rejected by pregnant mothers, speculate what protects the fetal allograft from being recognized as non-self. Do you think fetal cells lack MHC I?

Nude mice and humans with the mutation described by OMIM code 242700 lack T cells due to a nonfunctional thymus. T cells are called "T" because they mature and become "educated" to recognize self in the *t*hymus. Without a thymus and T cells, animals are doomed to die from viral infections since T cells are primarily responsible for this portion of our immunity. T cell receptors interact with the MHC I molecule more than the peptide in the example 1OGA, but the exact number of interactions varies with different T cell receptors and different peptides. The exact number of interactions is not important but what is important is that both MHC I and the peptide are in direct physical contact with the T cell receptor. Fetal cells must have MHC I molecules or the natural killer cells would attack and destroy the fetus. The best possible candidate for protecting the fetus seems to be the MHC I molecule itself since that is the one constant of all cells and protects the cells from natural killer cells. Are all MHC I alleles able to provide the fetus protection?

Before examining more data, reflect upon what you know from your life experience. You have probably heard about bone marrow donation and other organ donor programs but you may not have realized that these programs are trying to find MHC I allele matches between donors and recipients. Every day, 18 people die in the United States due to a lack of suitable donor tissue (see http://organdonor.gov/). The total human gene pool consists of about 2,000 MHC I alleles. Given that humans are diploid, the probability of two unrelated people matching MHC I alleles is approximately 1 in 4,000,000 which explains why it is so hard to find a good tissue match for human organ donation. You can learn more about human organ donation and tissue typing from the United Network for Organ Sharing (http://www.unos.org/) and the National Marrow Donor Program (http://www.marrow.org/). With regards to pregnancy, the odds are one in four million that a couple would have identical MHC I alleles and therefore you would expect the pregnant woman to reject her fetus because it is 50% non-self MHCH I. Furthermore, the 53 genes encoded on the mouse Y chromosome were sufficiently non-self to be rejected by female mice as summarized in Table 18.1. First-set and second-set skin allograft rejection occurred in female mice, but all these females were fertile and delivered healthy male and female offspring. How can every viable fetus produce a protective MHC I molecule?

In order to understand how the fetus is protected, you need to learn the relationship between the mother and fetus (Figure 18.8a). The fetus is 50% identical to the mother, but half of all the proteins produced by the fetus, including half of the MHC I molecules, are non-self. The amnionic sac that surrounds the fetus as well as the umbilical cord that brings nutrients and oxygen to the fetus are both fetal tissue. You can see that these two tissues do not directly interact with the mother's cells so you might predict they would be safe from T cell attack. However, notice that the umbilical cord moves its fluid content through the thousands of finger-like projections of the **trophoblast** tissue that is genetically fetal in origin. The blood-rich **endometrium** is 100% maternal tissue and it comes into direct contact with the fetal trophoblast and its constituent individual cells called **cytotrophoblasts**. Collectively, the trophoblast and the endometrium form the **placenta** which is a defining characteristic of all mammals that nurture their young internally until birth. Given its high blood content, the placenta is the exact site where you would expect the maternal immune system to attack fetal cells and thus kill the entire embryo. The key to an embryo's survival is in the MHC I alleles it expresses and presents on every cell derived from the 50% non-self fertilized egg. {*Definitions*: **Trophoblast** is the fetal tissue that physically interacts with the mother to transport nutrients and oxygen to the fetus. **Endometrium** is the blood-rich female tissue in the uterus that provides nutrients to the embryo

and is discarded every month during menstruation. **Cytotrophoblasts** are cells of the trophoblast tissue. **Placenta** is a mixture of fetal and maternal tissue composed of the trophoblast and the endometrium.}

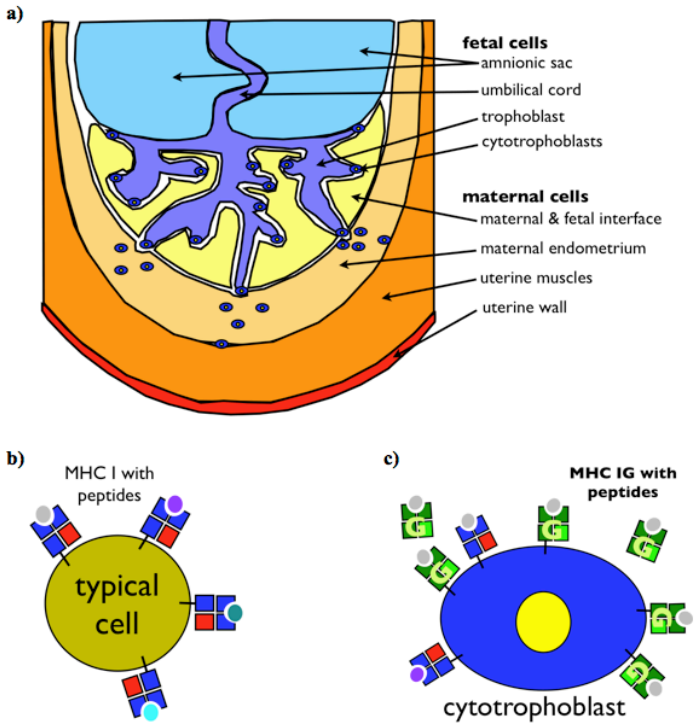


Figure 18.8 Maternal and fetal cells intermix during mammalian pregnancy. (a) Diagram of developing placenta below a fetus (not shown). (b) Cells use MHC I + peptide to identify themselves to immune cells. (c) Fetal cells also secrete and display peptides bound to unique MHC IG molecules.

When immunologists acquired the ability to sequence DNA, they very quickly wanted to know which genes were encoded in the MHC locus of the mammalian genome. {*Connections: The base pair sequence of DNA was covered in Chapter 1.*} The human MHC locus is a 4 million base pair region on chromosome 6p.21.3 and contains about 200 genes. In 1991, immunologists discovered a new MHC I gene called G, or MHC IG, within the overall MHC locus (Figure 18.8c). Once immunologists knew to look for MHC IG gene expression, the only place they could find it was in fetal cells. MHC IG molecules are very similar in shape to the other MHC I proteins and MHC IG molecules also present peptides from proteins made inside fetal cells. Through alternative splicing, some of the MHC IG proteins are not anchored to the membranes of fetal cells and they float freely in the area surrounding fetal cells. Maternal and paternal cells do not produce MHC IG proteins.

With the discovery of the MHC IG gene, immunologists had a very good candidate mechanism for fetal protection from the maternal immune system (Figure 18.9). Investigators used the very sensitive method of reverse transcriptase PCR (RT-PCR) to detect MHC IG mRNA produced inside recently fertilized human embryos. {*Connections: RT-PCR was described in Chapters 6 and 14.*} RT-PCR amplifies mRNA via the production of cDNA and does not amplify the genomic DNA because chromosomal DNA was destroyed at the beginning of the procedure. Manipulating RNA and tiny embryos is technically difficult but the investigators were able to detect MHC IG mRNA in the fertilized human embryos prior to implantation. But as you know, mRNA is not the functional molecule, MHC IG protein is, so a different group of immunologists wanted to detect the protein in slightly older embryos (Figure 18.9b). The investigators produced MHC IG-specific antibodies and covalently linked green dye to the antibodies. When embryos and the antibodies were mixed together, the investigators could detect MHC IG protein by the appearance of green tissue when viewed using fluorescence microscopy.

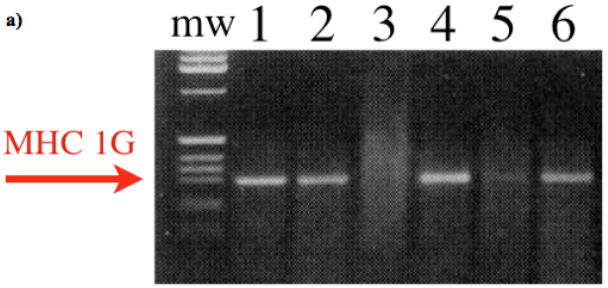
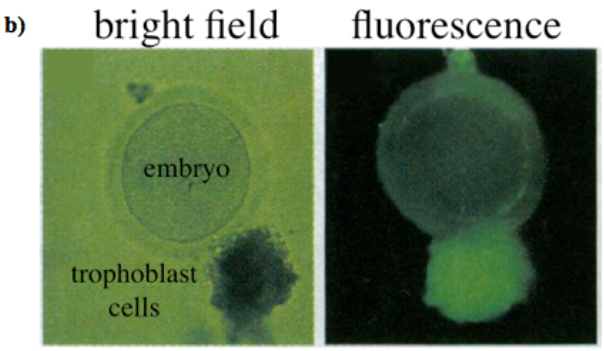


Figure 18.9 Detection of MHC IG in preimplantation embryos. (a) Six human embryos were tested for the



presence of MHC IG mRNA (red arrow) using RT-PCR and analyzed by gel electrophoresis. (b) One human embryo from a fertility clinic was tested for MHC IG by fluorescent antibody labeling (green).

Detecting a protein by antibody binding does not definitively demonstrate MHC IG is the protective molecule for embryos (Figure 18.10). What was needed was a functional test to determine if MHC IG actually prevents destruction of non-self cells. The investigators isolated trophoblast cells from placenta and tested their ability to withstand lysis when challenged by three different sources of natural killer cells. The trophoblast sample was divided into three aliquots and a different set of antibodies was added to each aliquot. One portion of trophoblast was incubated with an arbitrary antibody that did not bind to any human protein. Another portion was incubated with antibody that bound specifically to human MHC IG while the final portion was incubated with an antibody that bound to MHC I but not MHC IG. Three sources of natural killer cells were incubated separately with the three portions of trophoblasts pretreated with antibodies for a total of nine tubes of cells and antibodies. The immunologists used a biochemical assay to determine what percentage of the trophoblast cells were attacked and lysed by the natural killer cells.

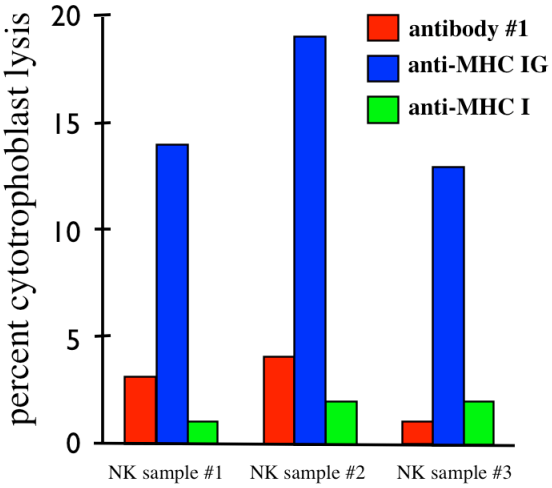


Figure 18.10 Challenging trophoblast cells with natural killer (NK) cells from unrelated individuals. NK cells from three different people added to separate samples from a common population of cytotrophoblasts. Antibodies were added to cytotrophoblasts prior to adding NK cells and measuring lysis. Standard deviation was less than 5% in triplicate experiments.

Integrating Questions

14. Search the nucleotide database at NCBI using the accession number "NM_002127.5". What is the chromosomal position of MHC IG? How many exons are in this gene?
15. Which portion of the preimplantation embryo in Figure 18.9b displayed the greatest concentration of MHC IG molecules on their surface? Why does this expression pattern of MHC IG make sense given the anatomy of a fetus inside the uterus?
16. Which antibody caused the most trophoblast cells to by lysed by natural killer cells in Figure 18.10? What do these results indicate about the relative abundance of MHC I vs. MHC IG molecules on the surface of trophoblast cells? Why weren't 100% of the cells lysed when incubated with the MHC IG antibodies?

MHC IG is within the MHC locus at chromosome position 6p21.3 and the gene is composed of eight exons, though only the first six exons encode for amino acids while the last two are non-coding. Based on the data in Figure 18.9b, trophoblast cells produce more MHC IG than does the

small circular embryo, as indicated by the brighter green cytotrophoblasts. Based on the natural killer cell lysis data in Figure 18.10, only MHC IG protects trophoblast cells from lysis which indicates these fetal cells display many more MHC IG proteins on their cell surfaces than the other MHC I proteins. Given that cytotrophoblasts directly interact with maternal natural killer and T cells, it makes sense that the trophoblast would benefit from a higher density of MHC IG than the fetus which is physically separated from the mother's immune system. The two irrelevant antibodies did not inhibit the protection offered to cytotrophoblasts but the anti-MHC IG antibody covered the protective MHC IG proteins which allowed the natural killer cells to attack the non-self cells. Only about 20% of the cytotrophoblasts were lysed when incubated with anti-MHC IG because there are so many MHC IG molecules that the antibodies could not cover all of them and so 80% were still protected from natural killer cell lysis.

A common theme in biology is that if a particular function is vital for survival, then individuals usually have more than one way to perform the function. {*Connections: Redundancy of critical functions was addressed in Chapter 1 about multiple DNA polymerase genes.*} Ensuring that a mother's immune system does not reject her fetus is a vital function and thus the fetus has more than one way to protect itself (Figure 18.11). If you search WileyPlus for **interleukin 10** (IL10), you will see this small molecule is produced by white blood cells and reduces inflammation and suppresses immune reactions. It might seem odd for white blood cells to make an immunosuppressive molecule, but IL10 is a form of negative feedback to stop overzealous immune responses from developing into autoimmune diseases. A group of immunologists wanted to know if trophoblast cells produced IL10 so they used antibody labeling to find out (Figure 18.11a). You can detect the presence of IL10 by the appearance of the dark stain on fetal cytotrophoblasts. A different immune system signaling molecule, IL7, was used as a negative control in their experimental design. The same team of investigators quantified the amount of human IL10 produced by trophoblasts at different stages during the pregnancy (Figure 18.11b). Although the data varied from sample to sample, the immunologists documented a clear trend in the amount of IL10 produced by cytotrophoblasts. {*Definition*: **Interleukin 10**, **IL10**, is a small molecule produced by white blood cells to reduce immune responses.}

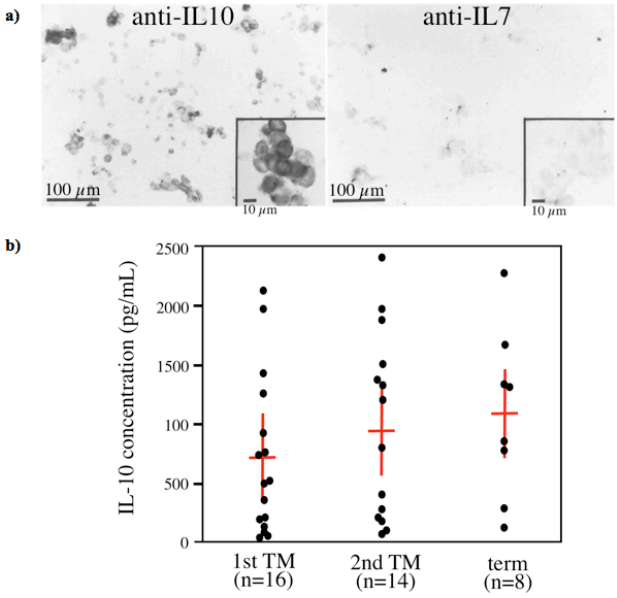


Figure 18.11 Measuring IL-10 levels in trophblast cells. (a) Cytotrophoblasts from first trimester (TM) labeled (dark color) with antibodies against either IL-10 or IL-7. (b) Quantification of IL-10 from cytotrophoblasts isolated during first and second trimesters, as well as at birth. Red bars represent averages ± 95% confidence interval.

In 1944, Medawar published his ground breaking paper in which he proposed that a healthy immune system was the cause of allograft rejection. By 1994, immunologists had a very solid working model to explain how the immune system distinguished self from non-self. Every cell uses MHC I molecules to display self-peptides. T cells scan all cells to find and destroy any tissue that appears to be non-self or virally infected cells. And by 2004, immunologists had

finally clarified the apparent contradiction of allograft rejection but maternal acceptance of a fetus. MHC IG and IL10 are two of the protective mechanisms employed by embryos to prevent their destruction. One benefit of having a working model is it allows you to make testable predictions. For example, you would predict that mutations causing the loss of MHC IG would be very rare. Furthermore, you would predict that any new mutations in sperm or egg that caused a loss of MHC IG should result in spontaneous abortions and sterility.

Integrating Questions

17. Based on the data in Figure 18.11, do cytotrophoblasts produce IL10? Do they produce IL7? Describe the general trend of IL10 production over the three trimesters of human pregnancy.
18. Use a medical dictionary website to define the terms preeclampsia and eclampsia. What medical conditions do these two terms describe?
19. Search PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) using the three author names of Hylenius, Melbye and Hviid to locate a 2004 paper they and others published in *Molecular Human Reproduction*. This paper is freely available so you can download it free of charge. What gene and mutation appear to be responsible for at least some forms of preeclampsia?

It is clear from the data in Figure 18.11 that fetal cells produce IL10 just as they produce MHC IG. As the pregnancy progresses, the trophoblast appears to produce more IL10 which may help ensure the maternal immune system does not become more aggressive and override the initial concentration of IL10. Remember from Figure 18.1b that the more allograft cells presented to the recipient immune system, the more aggressive the immune reaction. From your understanding of maternal tolerance of a non-self fetus, you can predict that genetic deficiencies in MHC IG should lead to immune rejection and spontaneous abortion. The term **preeclampsia** describes a serious medical complication for the mother due to her pregnancy. The expectant mother experiences high blood pressure and protein in the urine that typically develops after the 20^{th} week of the pregnancy. **Eclampsia** is life-threatening condition when the mother experiences convulsions, or seizures, due to high blood pressure brought about by the pregnancy. Do these conditions have anything to do with maternal tolerance of non-self fetal cells? {Definitions: **Preeclampsia** is a pregnancy-induced medical condition that results in protein in the urine due to high blood pressure. **Eclampsia** is a life-threatening medical condition brought on by pregnancy-associated high blood pressure and no other cause.}

Hylenius and colleagues documented the correlation between particular MHC IG alleles and preeclampsia (Figure 18.12). The human MHC IG gene is composed of 8 exons with the last two exons being part of the **3' untranslated region** of the mRNA. {*Connections: Chapter 2 addressed the presence of nucleotides in mRNA after the stop codon.*} Many students think of the untranslated regions of mRNA as not having a function, but as you can see from this example, 3' untranslated regions do have a function. One allele of MHC IG contains 14 bp within exon 8 while another allele lacks these 14 base pairs. Hylenius and her Danish colleagues studied 155 families and found that 30% of preeclampsia fetuses had a genotype of +14 bp/+14 bp while only 7% of control fetuses had the same genotype. When parents were heterozygous for the 14 bp allele, the father passed on his +14 bp allele 70% of the time in preeclampsia fetuses while the mother passed on the +14 bp allele only 23% of the time in control fetuses ($p < 0.006$). {Definition: **3' untranslated region** of a gene refers to the nucleotides downstream of the stop codon that is transcribed and becomes part of the mRNA.}

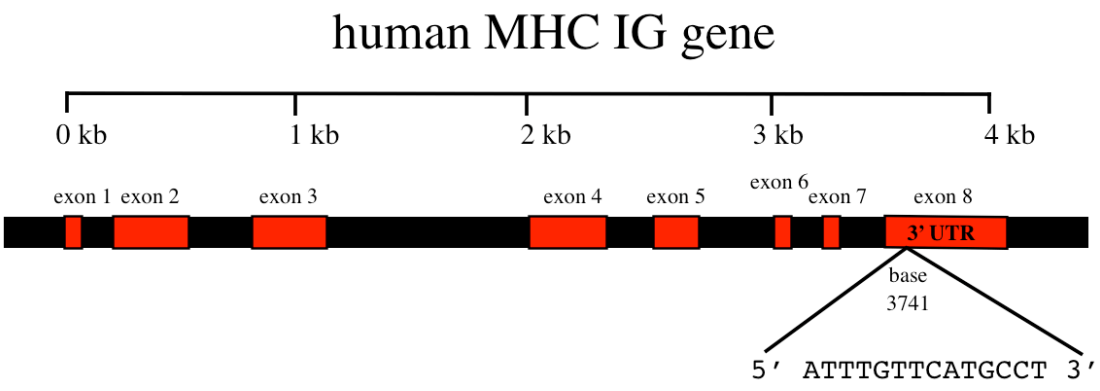


Figure 18.12 Structure of human MHC IG gene. The gene is about 4 kilobases long and composed of 8 exons. Fourteen bases are present or absent in different alleles of the gene.

Integrating Questions

20. Describe the two major types of MHC IG alleles depicted in Figure 18.12. What effect does this mutation have on the protein structure of MHC IG?
21. Based on the quantified data presented in the discussion of MHC IG alleles, is the MHC IG allele with the 14 bases or the allele lacking the 14 bases associated with preeclampsia? Given that not all of the preeclampsia fetuses had the same 14 bp MHC IG genotype, do you think MHC IG is the *only* cause of preeclampsia? Hypothesize a mechanism to explain why the paternal 14 bp mutation is more frequently associated with preeclampsia than the maternal source of the same allele.

A mother's immune system must be avoided for the fetus to go full term. When the fetus is unable to produce sufficient amounts of MHC IG due to a +14 bp insertion mutation in the non-coding region of the mRNA, the mother more frequently develops preeclampsia and the fetus is lost. Based on the data collected by Hylenius on Danish couples, the father's +14 bp allele appears to cause preeclampsia more frequently than the same allele from the mother. When you see a pattern where the source of the DNA and not the sequence is a critical factor, you should suspect epigenetic causes of the phenotype. {*Connections: Epigenetics was first introduced in Chapter 1.*} Presumably, just as there are redundant mechanisms to protect the fetus from the maternal immune system, there are probably multiple genetic causes of preeclampsia with MHC IG +14 bp allele being only one of them.

This Section began with two apparent contradictions which was the focus of the emergent property at the individual level – tolerance of mammalian pregnancy. If you studied only the immune system, you would not have predicted that a non-self fetus could be tolerated. Conversely, if all you studied was reproduction, you would not have predicted allograft rejection and how the immune system identifies and rejects non-self tissue. The emergent property of mammalian pregnancy exhibits competition between tolerance and immune rejection as well as cooperation within the placenta. MHC I proteins and their associated peptides are combined by random processes inside every cell in your body, which helps the immune system survey your cells for the potential of viral infection. In the next Section, you will examine a different type of definition for self and the individual.

References

Maternal Tolerance of Fetus

Bainbridge, David R. 2000. Evolution of mammalian pregnancy in the presence of the maternal immune system. Reviews of Reproduction. Vol. 5: 67 – 74.

Friedman, Eli A., Walden Retan, David C. Marshall, et al. 1961. Accelerated skin graft rejection in humans preimmunized with homolgous peripheral leukocytes. Journal of Clinical Investigation. Vol. 40: 2162 – 2170.

Friedman, Eli A., Carlo Valenti and R. Keith Waterhouse. 1964. "Accelerated" first-set rejection of skin homografts in the rabbit. Annals of the New York Academy of Sciences. Vol. 120: 140 – 149.

Gompertz, B. (1825). "On the Nature of the Function Expressive of the Law of Human Mortality, and on a New Mode of Determining the Value of Life Contingencies". *Philosophical Transactions of the Royal Society of London* **115**: 513–585. http://visualiseur.bnf.fr/Visualiseur?Destination=Gallica&O=NUMM-55920.

Hylenius, Sine, Anne-Marie Nybo Anderson, et al. 2004. Association between HLA-G genotype and risk of pre-eclampsia: a case-control study using family triads. Molecular Human Reproduction. Vol. 10: 237 – 246.

Jurisicova, Andrea, Robert F. Casper, et al. 1996. HLA-G expression during preimplantation human embryo development. PNAS. Vol. 93: 161 – 165.

Katsh, Grace F., David W. Talmage and Seymour Katsh. 1964. Acceptance or rejection of male skin by isologous female mice: Effect of injection of sperm. Science. Vol. 143: 41 – 42.

Lanman, Jonathan T., Jenny Dinerstein and Senih Fikrig. 1962. Homograft immunity in pregnancy: lack of harm to the fetus from sensitization of the mother. Annals of the New York Academy of Sciences. Vol. 99: 706 – 716.

Le Bouteiller, Phillippe, Corinne Solier, et al. 1999. In Mini symposium: The Major Histocompatability Complex in Pregnancy: Part II. Placental HLA-G protein expression *in vivo*: where and what for? Human Reproduction Update. Vol. 5(3): 223 – 233.

Medawar, P. B. 1944. The behaviour and fate of skin autografts and skin homografts in rabbits. (A report to the War Wounds Committee of the Medical Research Council). Journal of Anatomy. Vol. 78: 176 – 199.

Moreau, Philippe, Francisco Adrian-Cabestre, et al. 1999. IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. International Immunology. Vol. 11(5): 803 – 811.

Roth, Iris, David B. Corry, et al. 1996. Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10. Journal of Experimental Medicine. Vol. 184: 539 – 548.

Rouas-Freiss, Nathalie, Rachel Marchal-Bras Gonçalves, et al. 1997. Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. PNAS. Vol. 94: 11520 – 11525.

Speiser, Daniel E., Urusula Stübi and Rolf M. Zinkernagel. 1992. Extrathymic positive selection of αβ T-cell precursors in nude mice. Nature 355:170 – 172.