Organ donation and transplant: HLA typing, immunology & genetics extension

This resource is geared towards senior secondary science students. It is meant to serve as a "deep dive" into some of the immunology and genetics concepts that relate to organ donation and transplantation. It is a learning resource that can be discussed as a group, or worked through individually. It will hopefully serve as a launch-pad for further student inquiry (see "Inquiry Activity" at the end).

Warm-up questions:

- What is a gene?
- What do we already know about the immune system?
- The prefix poly- originates from the ancient Greek word for "many" (or "multiple"). For example, a polygon has "many" sides and angles. A polyglot is someone who speaks "many" languages. A polysyllabic word has "many" syllables in it. The suffix -morphic originates from the ancient Greek word that refers to the specific shape or form of something. With this information in mind, what do we think it means when a specific gene is described as "polymorphic"?

1. Gathering prior knowledge: important terminology

Work as a group to gather definitions for these terms, and any other information that feels relevant, based on student's prior knowledge and research.

Allele:

Antibody:

Antigen:

Gene:

Genome:

Leukocyte:

Organ transplant rejection:

Polymorphic:

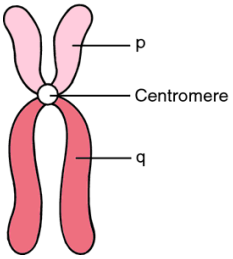
Protein:

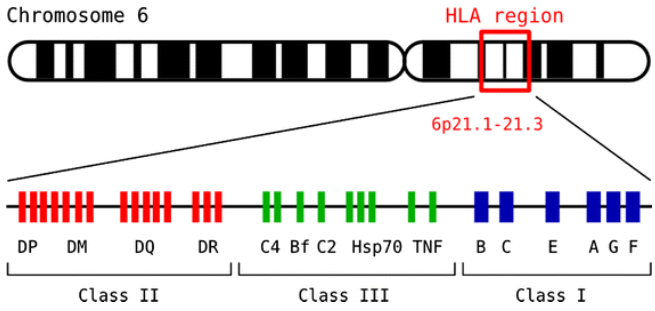
T cells:

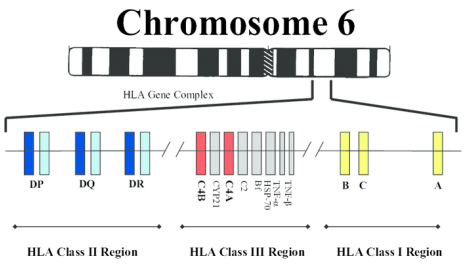
Tissue:

2. The Human Leukocyte Antigen (HLA) system

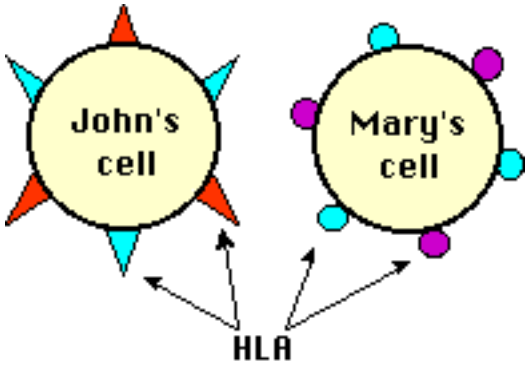
Human leukocyte antigen (HLA) genes are located on the short arm (called the "p" arm) of chromosome 6 (shown below).







HLA genes are responsible for encoding many different HLA **proteins**, which are found on the surface of human cells (see image below).



HLA proteins on the surface of human cells are recognized by specialized immune cells called **T cells**. The interaction between **HLA proteins on the cell surface** and **T cells of the immune system** teaches the immune system to recognize "self" vs. "non-self" (or *foreign*) cells and tissues. Cells are determined to be "self" or "non-self" based on the types of HLA proteins found on the cell surface. When T cells recognize "non-self" ("foreign") cells, they typically trigger an immune response to destroy these cells.

Every person has their own unique set of HLA genes, which encode a unique set of HLA proteins. Therefore, each person's immune system is going to have a different understanding of what is "self" tissue, and what is "non-self" (or *foreign*) tissue (based on that person's unique set of HLA genes and proteins). Each person's unique set of HLA genes can be referred to as their "HLA type".

*Note about terminology: **human leukocyte antigen (HLA**) is the human-specific name for the **major histocompatibility complex (MHC)**. If we were talking about mice or cockroaches (or some other non-human animal), we could call them "MHC genes". In humans, we can call them "HLA genes". These genes serve the same immune function in most animals (including humans) [that is, distinguishing between "self" and "non-self"].*

Think-Pair-Share:

- Based on what has been covered so far, why do you think HLA type might be relevant to the process of organ transplantation?

If everyone had an identical set of HLA genes, an organ from any donor could theoretically be "accepted" by the immune system of any recipient. However, HLA genes are the most **polymorphic** gene cluster of the entire human **genome**. In other words, there are many different versions and variations of each HLA gene within the human population. We refer to these gene variations as **"alleles"**.

As of June 2021, there are currently 31,552 HLA and related alleles that have been named and included in the database of these alleles.

The polymorphic nature of HLA genes means that there can be large variations in the types of HLA proteins present on the surface of each person's cells. This presents a challenge when it comes to organ transplantation.

After an organ transplant, the recipient's immune system will interact with the new organ to determine whether it is a "foreign" tissue that it should attack. If the HLA proteins on the cells of the donated organ are similar to the HLA proteins on the recipient's other cells (non-transplanted cells), the recipient's immune system is more likely to **accept** the new organ. If there is a large discrepancy between donor and recipient HLA proteins, it is more likely that the organ will be rejected.

Think-Pair-Share:

- The first successful human organ transplant took place in 1954, when a kidney was transplanted between two identical twins. In genetic terms, why was the kidney not rejected in this situation?

If a donor and recipient share the exact same set of HLA genes, the recipient's immune system is unlikely to reject the transplanted organ because the recipient's immune system will recognize the new organ as "self" tissue based on the similarity of HLA type. However, as the number of mismatched ("different") HLA alleles between the donor and recipient increases, it becomes less likely that an organ transplant will be successful because the recipient's T cells will recognize the donor's mismatched HLA proteins as "non-self" and reject the organ.

When determining whether an organ transplant is likely to be successful, HLA type is considered. Medical professionals want to be confident that the transplanted organ is not going to be rejected by the recipient's immune system.

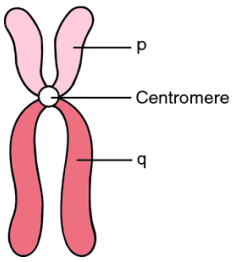
3. What makes a "match"?

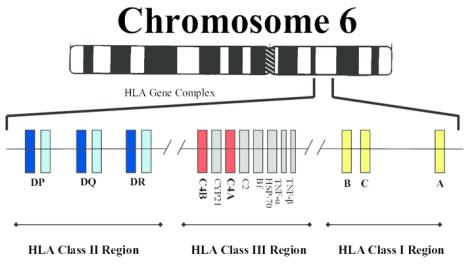
The entire HLA gene cluster is a very complex system, and it is not entirely understood by scientists and medical professionals. For simplicity's sake, let's focus on the classical transplantation HLA genes, the HLA class I genes: HLA-A, HLA-B, HLA-C.

Because we have 1 copy of each of these genes (HLA-A, HLA-B, and HLA-C) on each p arm of our sixth chromosome, we will have a total of 2 copies of each (one for each p arm, one from our biological mother and one from our biological father). This means that each person will have a total of **six** alleles of these classical transplantation genes.

The allele types are **numbered**—so different alleles of HLA-C, for example, would be called HLA-C*1, HLA-C*2, HLA-C*3, HLA-C*4, etc. (note the different numbers).

Thousands of HLA-A, HLA-B, and HLA-C alleles have been reported. The number of possible variations and combinations can feel a bit mind-boggling!





*Because we have 2 copies of chromosome 6 (we inherit one from our biological mother, and one from our biological father), we each have two copies of HLA-A, HLA-B, and HLA-C, for a total of **six** alleles of these classical transplantation HLA genes.*

To be an "HLA match", the donor and recipient would need to have the same allele for each of the six classical transplantation HLA genes. In reality, it is more complex than this, but for the purposes of this learning resource, we are keeping it more straightforward. Feel free to inquire further about this complexity if you are interested.

Example of an "HLA match":

Donor	HLA-A*3	HLA-A*24	HLA-B*7	HLA-B*49	HLA-C*6	HLA-C*24
Recipient	HLA-A*3	HLA-A*24	HLA-B*7	HLA-B*49	HLA-C*6	HLA-C*24

Because this donor and recipient have the same **alleles** or **variants** for all six of the classical transplantation HLA genes, we could consider them to be an "HLA match".

4. Determining HLA type in the medical laboratory

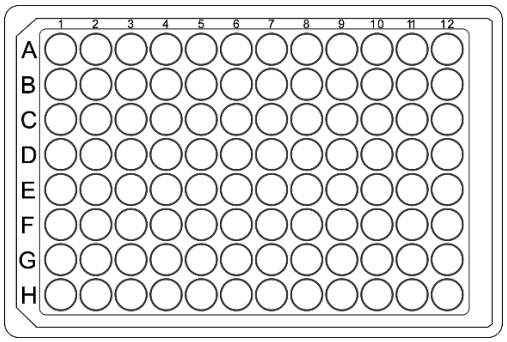
HLA type can be determined **serologically** (in other words, through blood testing) or **molecularly** (in other words, through DNA testing).

The goal of any HLA typing experiment is to determine which specific HLA genes a particular person has.

Serological (Blood) Testing

Let's focus on serological testing first. Imagine that you have a sample of a patient's blood, and you want to know the HLA type. In other words, you want to know which HLA proteins are present on the surface of these blood cells.

To figure this out, you would need a "well plate"–a plastic plate with many different "wells". The well plates shown below have 96 wells.



For this type of test to work, each well needs to contain a different **antibody** that is known to bind to **only one** specific HLA protein (i.e., the product of **one** specific HLA allele). Antibodies that bind specifically to certain HLA proteins can be purchased from scientific/medical supply

companies. Once you have your different antibodies loaded into each well (**one type of antibody per well**), blood cells from the patient's blood sample can be added.

Now, each well contains:

- an antibody that binds specifically to one type of HLA protein
- a sample of blood cells from a patient

In this type of test (called a microcytotoxicity assay), successful binding between the antibody and its specific HLA protein partner will cause the patient's blood cells to be stained pink (this is a "positive" test result). If binding does not occur, the cells will not be stained pink (this is a "negative" test result). Once the reaction has had enough time to incubate, you can use a microscope to determine which wells contain cells that have been stained pink.

If a cell is stained pink, you can conclude that the antibody in that well was able to bind to its specific HLA protein "partner", indicating that this HLA protein must be present on the surface of the patient's blood cells. By examining each well using microscopy, you could determine the patient's HLA type by recording which antibodies resulted in a positive test result (successful binding—cells stained pink—indicating that their HLA protein binding partner was present on the surface of the patient's blood cells).

Molecular (DNA) Testing

To determine HLA type via DNA testing, polymerase chain reaction (PCR) technology is utilized. View this video about PCR technology: https://youtu.be/c07_5BfIDTw

Video citation: miniPCR bio. (2020, February 15). What is PCR? Polymerase Chain Reaction [Video]. YouTube.

For our PCR-based HLA typing experiment, we begin with another well plate (see images above). Each well will contain a different **primer** that is complementary to a specific HLA allele sequence. Once each well contains a different primer (one type of primer per well), a sample of the patient's DNA is added to each well.

If the patient's DNA contains the HLA allele sequence that is complementary to the specific primer in the well, this primer will successfully bind to the patient DNA, and the region of DNA containing this specific HLA allele will be amplified by PCR technology.

In other words, DNA will be amplified in wells where the primer matches up with one of the patient's specific HLA alleles.

From here, the amplified DNA can be examined via **gene sequencing** or **gel electrophoresis**, which will allow us to know which HLA alleles are present in this patient's cells (and, therefore, allows us to determine the patient's HLA type).

Video about gene sequencing: https://youtu.be/rA8MUR4pqNE

Video citation: University of New South Wales. (2020, February 24). How does a DNA sequencing machine work? [Video]. YouTube.

Video about gel electrophoresis: https://youtu.be/GUXKQBknYQo
Video citation: miniPCR bio. (2020, December 17). What is Gel Electrophoresis? [Video]. YouTube.

5. Inquiry activity

What questions came up for you as you went through this information about HLA typing, genetics, and immunology?

Create a letter-sized (8.5"x11") poster highlighting some important information that relates to your question. Incorporate information from at least 3-5 reputable sources into your poster. Use the Note-Taking Sheet to keep track of your sources, if desired.

Example inquiry questions:

1. What is the pathway from HLA gene to HLA protein?
2. How does polymerase chain reaction (PCR) technology work?
3. How are HLA genes inherited?

4. Even if a patient has been determined to be an HLA match for a particular organ, they may show signs of transplant rejection after receiving the organ. What symptoms would be classified as evidence of acute organ rejection? In this case, what would doctors do to treat this acute organ rejection? Why do these treatments make sense?

6. Additional resources and further reading (student-friendly)

Video about HLA tissue typing: https://youtu.be/Pc8e68Wm7bs
Schrader, B. (2015, April 21). Tissue Typing [Video]. YouTube.

- This video also discusses laboratory methods for HLA matching, including HLA antibody screening and cross-matching (which were not covered in this resource)

Video about cross-matching in a Canadian hospital laboratory:
https://youtu.be/DmNO3adbLUw
Canadian Society for Medical Laboratory Science. (2017, March 6). In The Lab – Cross Matching [Video]. YouTube.

Article outlining HLA inheritance (how HLA genes are passed down from parents to children):
https://web.stanford.edu/dept/HPST/transplant/html/hla.html
Stanford University. HLA Matching, Antibodies, and You.

References: HLA Typing, Immunology, and Genetics Extension

This resource was created using information from the following resources.

- *poly-* word etymology: https://membean.com/wrotds/poly-many
- *-morphic* word etymology: https://membean.com/wrotds/morph-shape
- Drawing of chromosome 6: https://medical-dictionary.thefreedictionary.com/chromosome+arm
- Map 1 of HLA complex (shown on chromosome 6): https://link.springer.com/article/10.1007/s00005-011-0137-y/figures/3
- Map 2 of HLA complex (shown on chromosome 6): https://www.researchgate.net/figure/A-simplified-map-of-the-HLA-region-on-human-chromosome-6_fig1_221914655
- Picture of John's cell and Mary's cell (showing HLA proteins on cell surface): https://web.stanford.edu/dept/HPST/transplant/html/hla.html
- 96-well plate image 1: https://www.biomat.it/product/microplates/hts-plates/96-well-plate-hts/
- 96-well plate image 2: https://commons.wikimedia.org/wiki/File:96-Well_plate.svg
- Information in the written text was gathered from:
    - Chapter 10: Major histocompatibility complex: Antigen processing and presentation: https://www.ncbi.nlm.nih.gov/books/NBK459467/
    - The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2628004/
    - Historical Overview of Transplantation: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3684003/
    - https://en.wikipedia.org/wiki/Histocompatibility
    - https://en.wikipedia.org/wiki/Transplant_rejection
    - Human Leukocyte Antigen Test: https://www.encyclopedia.com/medicine/divisions-diagnostics-and-procedures/medicine/human-leukocyte-antigen-test
    - Note about there being over 30,000 identified HLA alleles (as of June 2021): http://hla.alleles.org/alleles/index.html