An Introduction to Transplant Immunology

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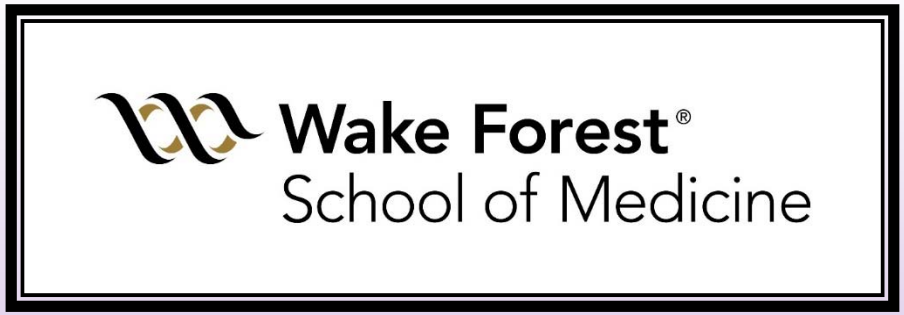


Objectives

- **Compare and contrast innate and adaptive immunity at a basic level.**
- **Understand basic concepts of transplantation.**
- **Appreciate the research that led to the discovery of transplant immunology.**
- **Appreciate the role of laboratory in upholding the principles of the Declaration of Istanbul.**

The First Transplant?

Sts. Cosmas and Damian c. 4th Century (modern-day Turkey/Syria)

Master of Los Bilases, c. 1495

Immunology

- **Relatively "new" science**
  - **1796**
- **Origin attributed to Edward Jenner**
  - **Invented vaccination (vacca)**
  - **Infected child with cowpox**
    - » **then with smallpox**
    - » **IRB won't approve**

Evolution of the Immune System

- Human species has gone through numerous phases in history characterized by different pathogen exposures
- Introduction of agriculture some 10,000 years ago made the spreading of new pathogens more likely
- The host-pathogen interaction is a very important relationship that serves to shape the immune system development early on in life.

Hygiene Hypothesis

- **Our immune system has been strongly focused on fighting off infections, even early in infancy.**
- **The hygiene hypothesis states that a lack of early childhood exposure to infectious agents, symbiotic microorganisms (such as the gut microbiome or probiotics), and parasites increases susceptibility to allergic diseases by suppressing the natural development of the immune system**
- **The lack of exposure is thought to lead to defects in the establishment of immune tolerance (Lost Friends)**

Overview of Transplant Immunology

- Innate and Adaptive Immunity
- Transplantation
- Brief History of Field
- Immune Response
- Declaration of Istanbul
- Wrap Up

Host Defense Systems

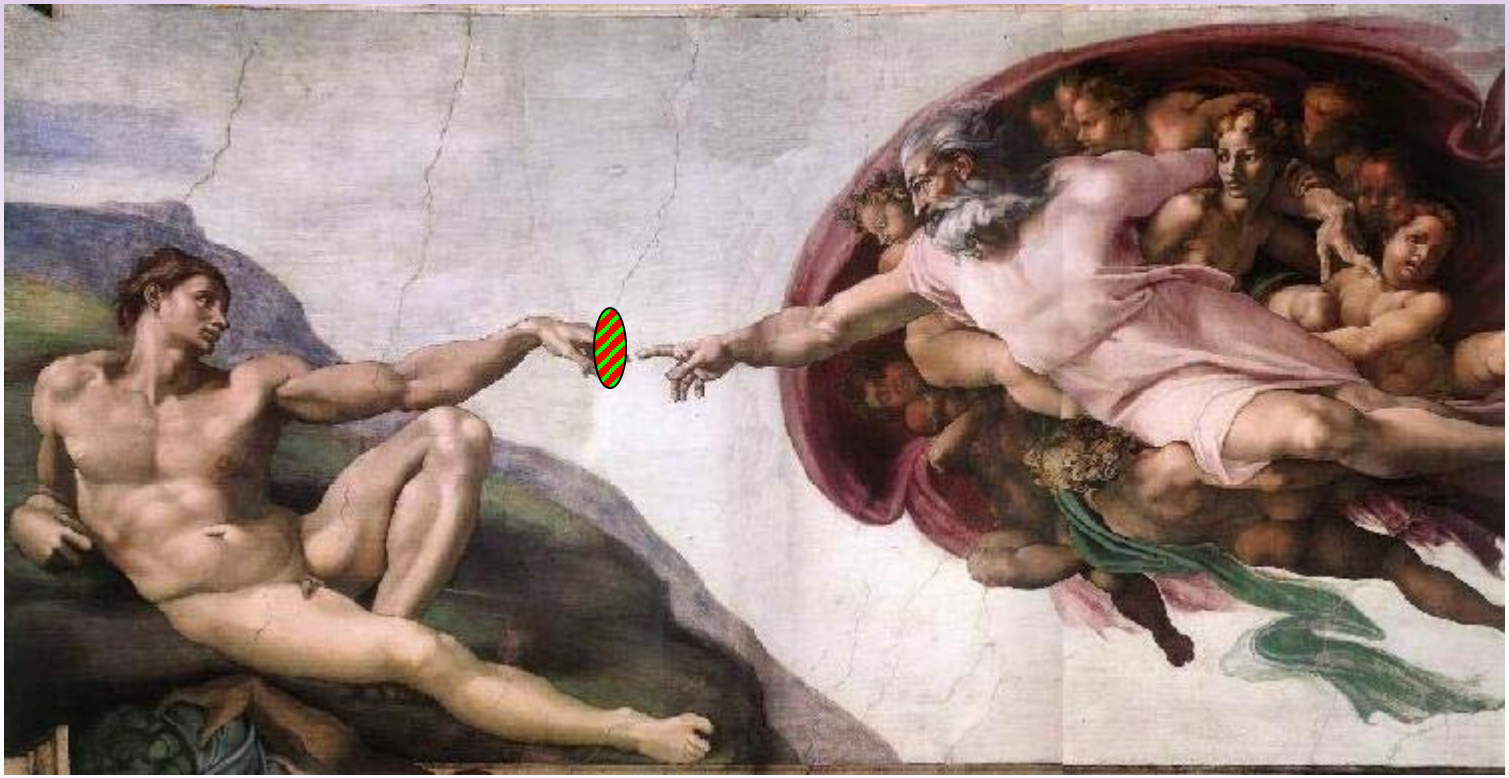
Innate

- external barriers (skin, mucus membranes)
- secretory components (enzymes, histamine, oxygen radicals, etc.)
- certain leukocytes (phagocytes, NK cells, platelets)
- no increase in strength after exposure

Adaptive (Acquired)

- evoked during an immune response
- T & B cells
- Leukocytes (monocytes, neutrophils, mast cells)
- Soluble factors (antibodies, cytokines)

The central component of the innate immune system is exclusion



HLA T-cell receptor

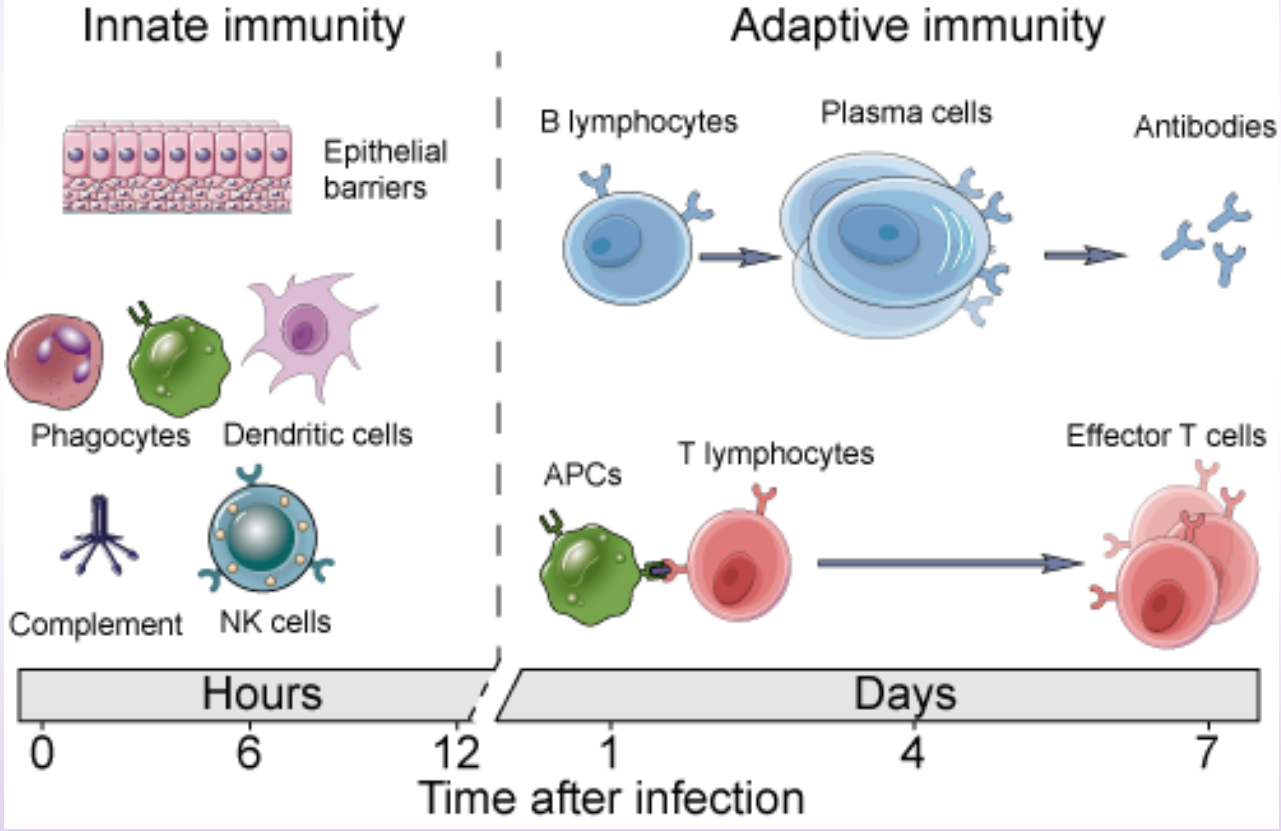
The central component of the adaptive immune response is the binding of peptide to HLA (MHC) and the recognition of the complex by T-cells

Innate and Adaptive Immunity

	Innate	Adaptive
Characteristics		
Specificity	For molecules shared by groups of related microbes and molecules produced by damaged host cells	For microbial and nonmicrobial antigens
Diversity	Limited; germline encoded	Very large; receptors are produced by somatic recombination of gene segments
Memory	None	Yes
Nonreactivity to self	Yes	Yes
Components		
Cellular and chemical barriers	Skin, mucosal epithelia; antimicrobial molecules	Lymphocytes in epithelia; antibodies secreted at epithelial surfaces
Blood proteins	Complement, others	Antibodies
Cells	Phagocytes (macrophages, neutrophils), natural killer cells, innate lymphoid cells	Lymphocytes

https://www.creative-diagnostics.com/innate-and-adaptive-immunity.htm

Timing of Responses



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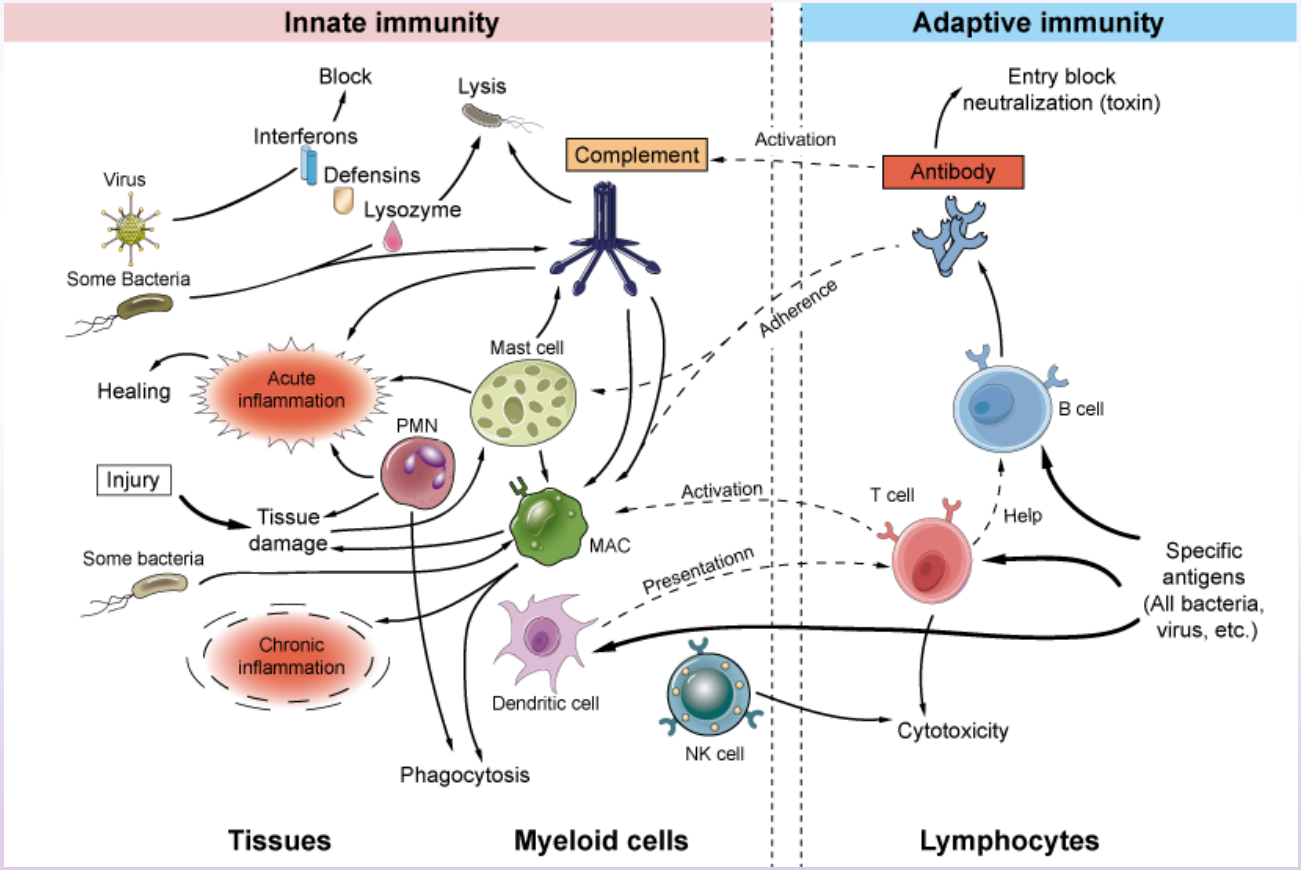
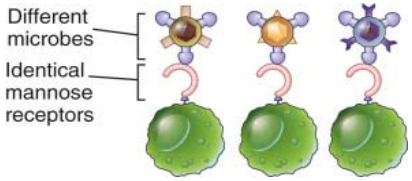
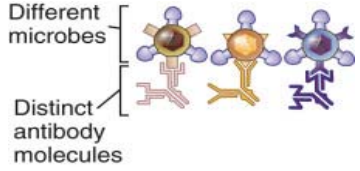
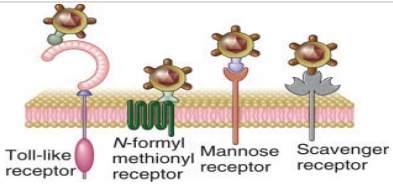
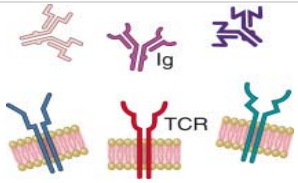
Shared and Unique Areas



https://www.creative-diagnostics.com/innate-and-adaptive-immunity.htm

Innate and Adaptive Immunity

	Innate Immunity	Adaptive Immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns) 	For structural detail of microbial molecules (antigens); may recognize non-microbial antigens 
# of molecules recognized	~1000 molecular patterns	>10^7 antigens
Receptors	Encoded in germline; limited diversity (pattern recognition receptors) 	Encoded by genes produced by somatic recombination of gene segments (greater diversity) 
# receptors	<100 different types of invariant receptors	Only 2 types of receptors (Ig and TCR), with millions of variations of each
Distribution of receptors	Nonclonal: Identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Genes encoding receptors	Germline encoded, in all cells	Formed by somatic recombination of gene segments only in B and T cells
Discrimination of self vs. non-self	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

Cellular and Molecular Immunology, Ninth Edition

Overview of Transplant Immunology

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A Brief History of Transplantation

- 1823: First skin autograft-transplantation of skin tissue from one location on an individual's body to another location (Germany)
- 1905: First successful cornea transplant by Eduard Zirm (Czech Republic)
- 1908: First skin allograft-transplantation of skin from a donor to a recipient (Switzerland)
- 1933: First successful cadaveric AB-0 incompatible kidney transplant (donor was B(III) and the recipient has 0(I)) by Yuriu Yu. Voronoy (USSR)
- 1950: First successful kidney transplant by Dr. Richard H. Lawler (Chicago, U.S.A.)[95]
- 1954: First living related kidney transplant (identical twins) (U.S.A.)[96]
- 1955: First heart valve allograft into descending aorta (Canada)
- 1962: First kidney transplant from a deceased donor (U.S.A.)
- 1965: Australia's first successful (living) kidney transplant (Queen Elizabeth Hospital, SA, Australia)
- 1966: First successful pancreas transplant by Richard Lillehei and William Kelly (Minnesota, U.S.A.)
- 1967: First successful liver transplant by Thomas Starzl (Denver, U.S.A.)
- 1967: First successful heart transplant by Christian Barnard (Cape Town, South Africa)
- 1981: First successful heart/lung transplant by Bruce Reitz (Stanford, U.S.A.)
- 1983: First successful lung lobe transplant by Joel Cooper at the Toronto General Hospital (Toronto, Canada)
- 1984: First successful double organ transplant by Thomas Starzl and Henry T. Bahnson (Pittsburgh, U.S.A.)
- 1986: First successful double-lung transplant (Ann Harrison) by Joel Cooper at the Toronto General Hospital (Toronto, Canada)
- 1995: First successful laparoscopic live-donor nephrectomy by Lloyd Ratner and Louis Kavoussi (Baltimore, U.S.A.)
- 1997: First successful allogeneic vascularized transplantation of a fresh and perfused human knee joint by Gunther O. Hofmann
- 1997: Illinois' first living donor kidney-pancreas transplant and first robotic living donor pancreatectomy in the U.S.A. University of Illinois Medical Center
- 1998: First successful live-donor partial pancreas transplant by David Sutherland (Minnesota, U.S.A.)
- 1998: First successful hand transplant by Dr. Jean-Michel Dubernard (Lyon, France)
- 1998: United States' first adult-to-adult living donor liver transplant University of Illinois Medical Center
- 1999: First successful tissue engineered bladder transplanted by Anthony Atala (Boston Children's Hospital, U.S.A.)
- 2000: First robotic donor nephrectomy for a living-donor kidney transplant in the world University of Illinois Medical Center
- 2004: First liver and small bowel transplants from same living donor into same recipient in the world University of Illinois Medical Center
- 2005: First successful ovarian transplant by Dr. P. N. Mhatre (Wadia Hospital, Mumbai, India)
- 2005: First successful partial face transplant (France)
- 2005: First robotic hepatectomy in the United States University of Illinois Medical Center
- 2006: Illinois' first paired donation for ABO incompatible kidney transplant University of Illinois Medical Center
- 2006: First jaw transplant to combine donor jaw with bone marrow from the patient, by Eric M. Genden (Mount Sinai Hospital, New York City, U.S.A.)
- 2006: First successful human penis transplant (later reversed after 15 days due to 44-year-old recipient's wife's psychological rejection) (Guangzhou, China)[97][98]
- 2008: First successful complete full double arm transplant by Edgar Biemer, Christoph Höhnke and Manfred Stangl (Technical University of Munich, Germany)
- 2008: First baby born from transplanted ovary. The transplant was carried out by Dr Sherman Silber at the Infertility Centre of St Louis in Missouri. The donor is her twin sister. [99]
- 2008: First transplant of a human windpipe using a patient's own stem cells, by Paolo Macchiarini (Barcelona, Spain)
- 2008: First successful transplantation of near total area (80%) of face, (including palate, nose, cheeks, and eyelid) by Maria Siemionow (Cleveland Clinic, U.S.A.)

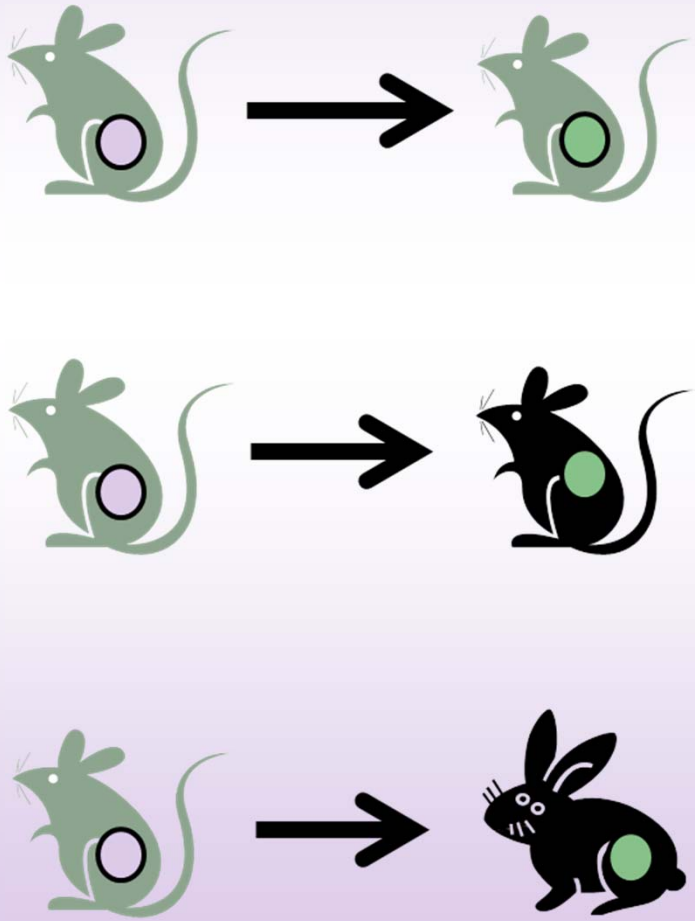
http://en.wikipedia.org/wiki/Organ_transplantation

Transplant Definitions

- **Transfer of an organ or tissue from one organism (donor) to another (recipient).**
  - **Skin, kidney, heart, lung, etc.**

- **Transfer of an organ or tissue from one site to another location in the same body.**
  - **CABG, rotationplasty (joint), bone, skin**
  - **Decellularized organ from donor re-seeded with autologous cells (tissue engineering)**

Types of Transplants



- **Autologous**
  - Autograft; transplant of cells or tissue to the same person
- **Syngeneic**
  - Isograft; transplant of organ or tissue from a donor to a genetically identical recipient
- **Allogeneic**
  - Allograft; transplant of organ or tissue to genetically non-identical donor of the same species
- **Xenogeneic**
  - Xenografts; transplantation of cells, tissues or organs from one species to another

Destinations

- **Orthotopic**
  - Transplantation of tissue from a donor into its normal position in the body of the recipient
  - Split organs may end up in 2 recipients
- **Heterotopic**
  - Transplantation of tissue typical of one area to a different recipient site
  - Kidney transplants most common
- **Extracorporeal**
  - Located or occurring outside the body

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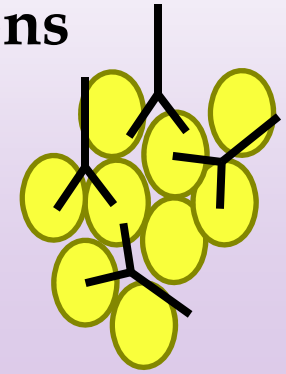
Historical Steps Elucidating Immune Response to Foreign Tissue

- Laboratory mouse used as a model system
- Early 1900s, researchers transplanting tumors in mice began to develop the "laws" of transplantation
- 1930s-1940s—Immunology meets genetics
  - Gorer identifies blood group locus encoding antigen II in mice
  - Snell independently maps gene controlling graft rejection to H (histocompatibility) locus
  - Both antigen II and H are the same yielding name "H-2" antigens
- Snell goes on to develop mouse strains to allow distinction of multiple histocompatibility genes, identify extensive polymorphism and complex genetics of system
- Klein, *Genetics*, 2001 vol. 159,p 435-439

Historical Steps Elucidating Immune Response to Foreign Tissue

- 1940s-1950s, Medawar, Burnet, Billingham begin to characterize immune response to foreign tissue and define immunologic tolerance using animal models
  - A History of Transplant Immunology, L. Brent, Academic Press 1997
- Studies of humans in 1950s and 1960s identify leukoagglutinins
  - Dausset observes alloreactive leukoagglutinins
  - Payne shows that pregnancy and transfusions induce leukoagglutinins



Groth et al. Historic Landmarks in Clinical Transplantation:… World J Surg 2000. 24:834-43.

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Innate Response

- **Initial tissue damage and stress leads to activation of the innate immune response**
  - **Mori et al. Immunol Reviews 2014. 258:132-144**
  - **Oberbamscheidt and Lakkis. Immunol Reviews 2014. 258:145-149**
- **Can cause damage through a variety of mechanisms**
- **Triggers adaptive immune response**
- **May bias the form that the adaptive immune response takes**

Discrimination of Self from Non-self

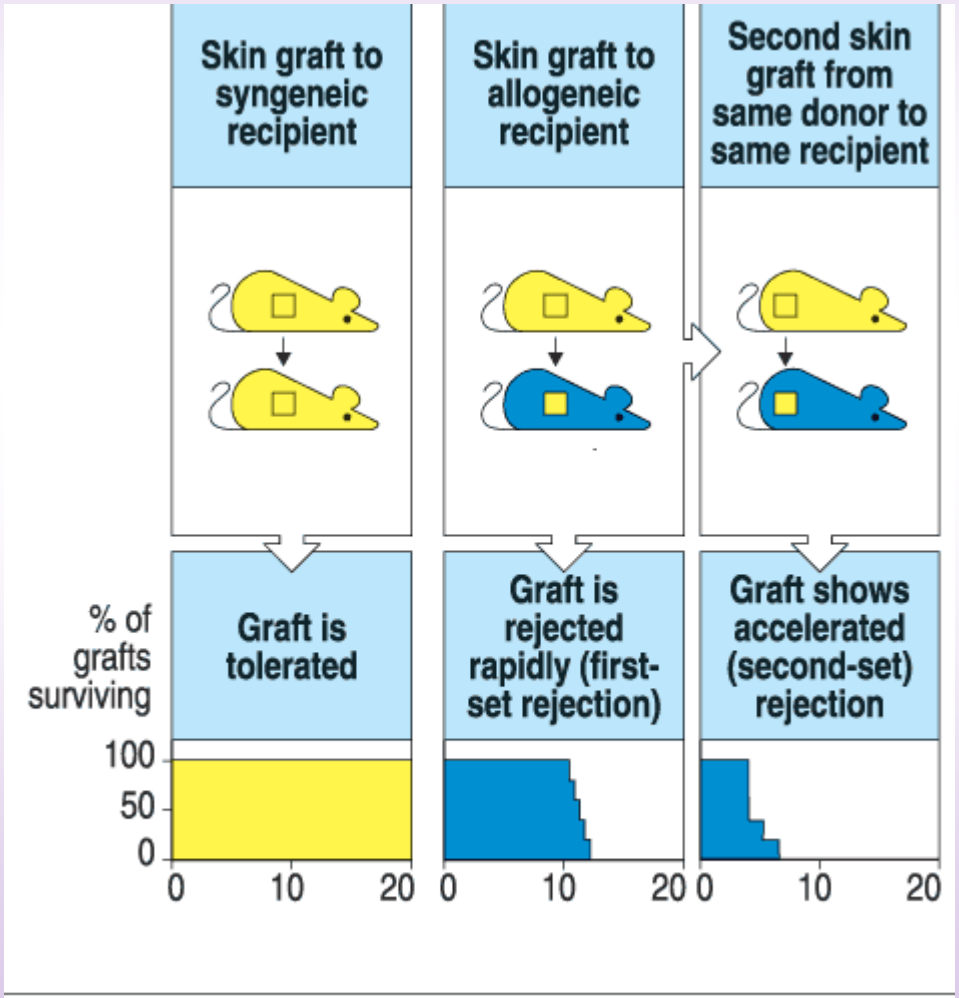
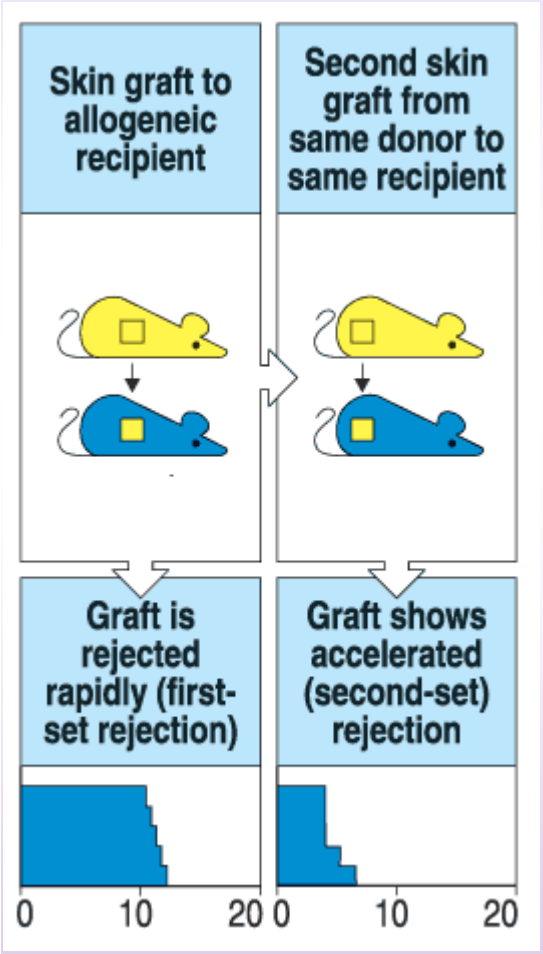
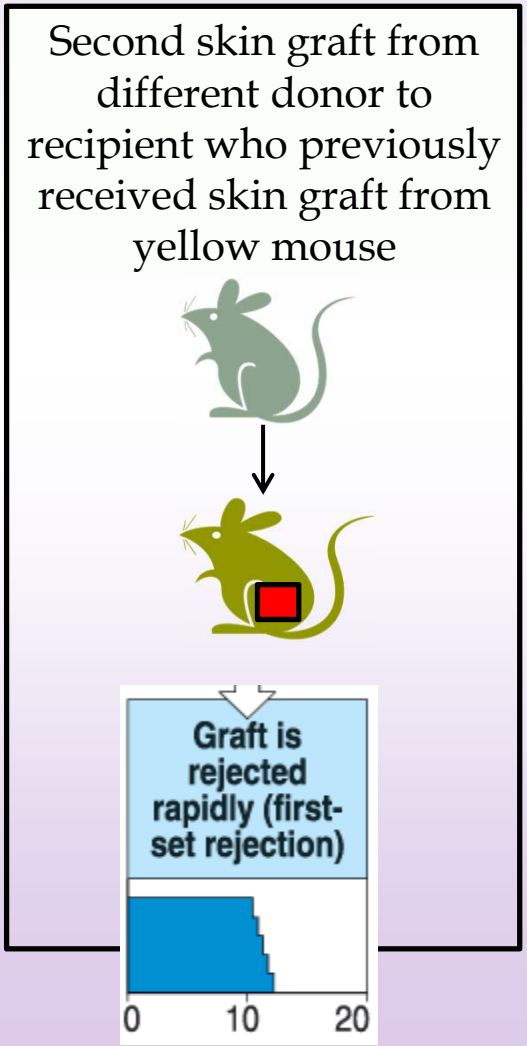


Fig 13.22 © 2001 Garland Science

- Self is "tolerated"
- Non-self (foreign tissue) is rejected
- Prior exposure to non-self causes stronger and more rapid rejection response (individual is "sensitized" to foreign tissue)

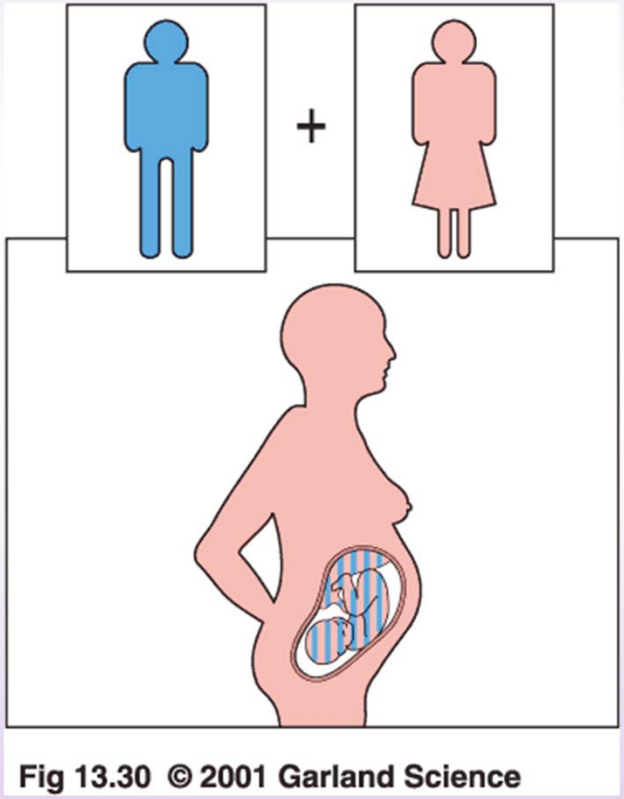
Immune Response Is Specific





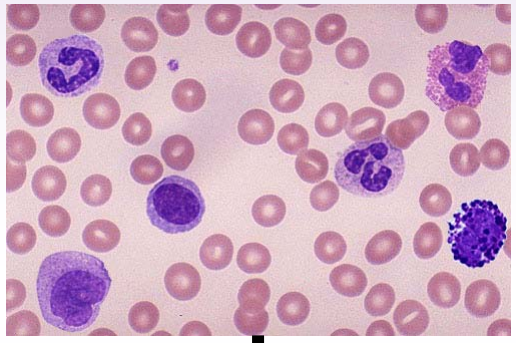
- **Recipient is not sensitized to tissue from the second donor**
- **Rejection to 2nd occurs based on timing of first exposure**

Human Response Is Through HLA

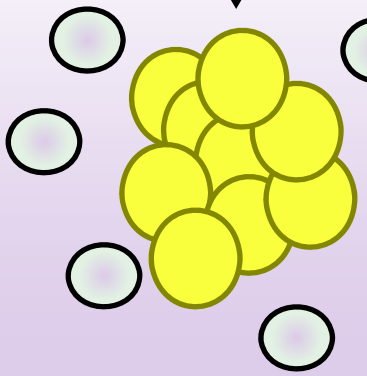


Sensitization through transplant, transfusion, pregnancy

Father's blood



Mother's serum

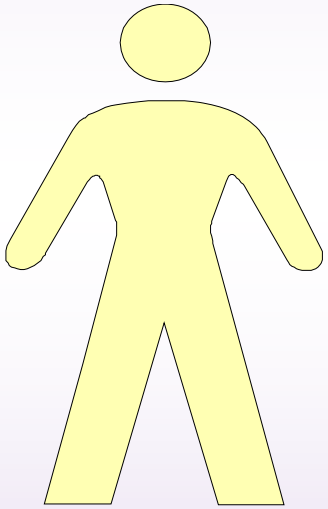


Agglutination of white blood cells (leukocytes)

Vector of Immune Response

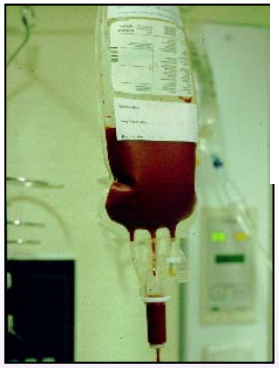
Patient (Recipient)

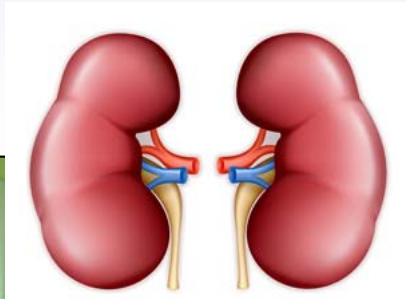
Graft (Donor)



GvHD

Rejection





Solid organ graft: Graft cells & possibly passenger leukocytes

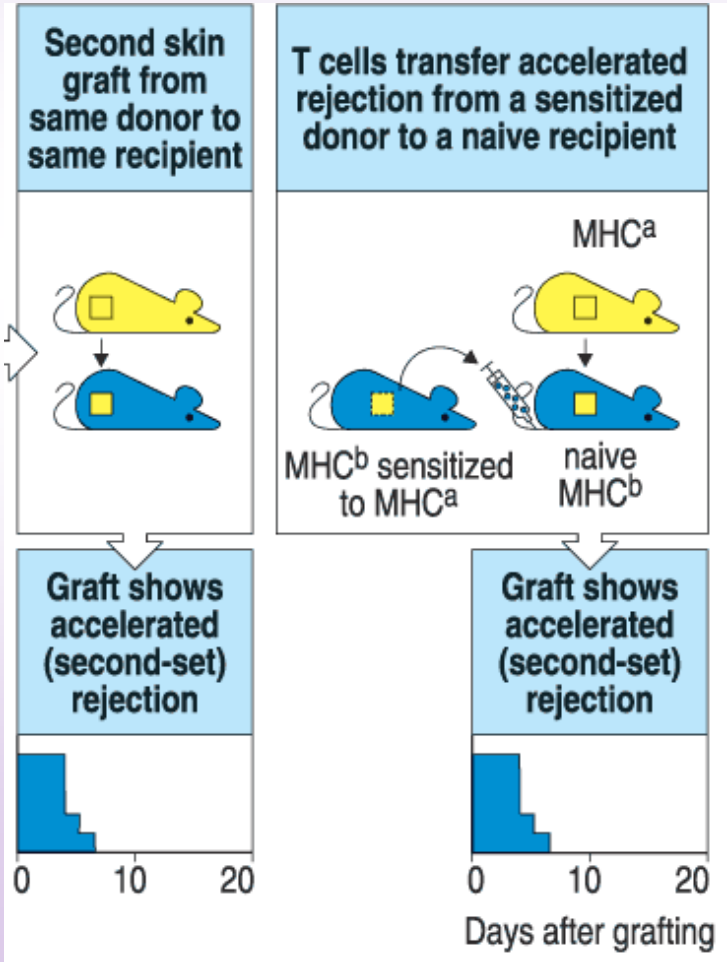
Possibly immune compromised or sensitized

Hematopoietic stem cell graft: Stem cells & immune cells

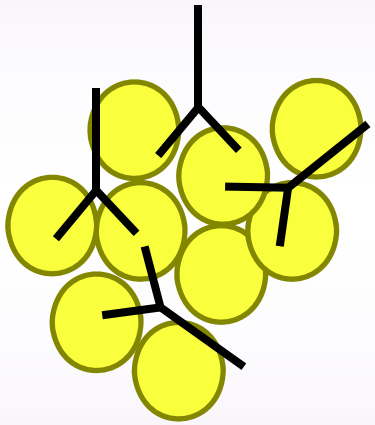
Platelet transfusion: Source of sensitization

Adaptive Immune Response to Alloantigens

Role of T Lymphocytes



Role of B Lymphocytes

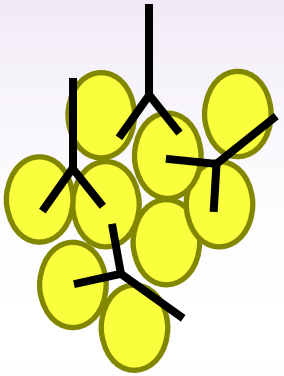


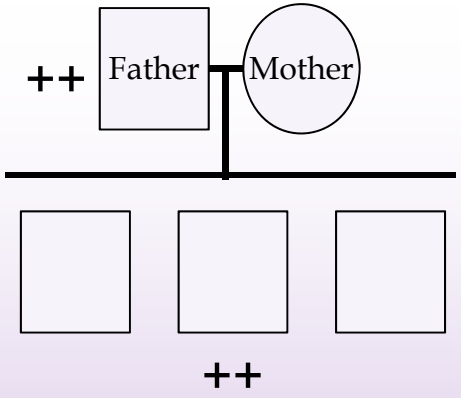
Agglutination: antibodies directed to cell surface antigens clump cells

- **Immune response to foreign tissue involves both T and B lymphocytes**
- **Response directed to major histocompatibility antigens**

Defining Human Leukocyte Antigen (HLA) Types

- **Dausset, Payne, Bodmer, Van Rood begin to define HLA types (MAC or LA (HLA-A2)), 4a and 4b (Bw4/Bw6)**
- **HLA types vary among individuals**
- **HLA types are inherited in families**





Mother's serum agglutinates cells from father and some of their offspring

	Leukocytes			
	Husband	Donor 1	Donor 2	Donor 3
Serum 1	++	++	--	--
Serum 2	++	++	--	++
Serum 3	++	--	++	--

Mother's serum agglutinates cells from father and some unrelated individuals

Sensitization to HLA Differences Can Result in Graft Rejection

- Individuals sensitized to foreign tissue make antibodies to the specific HLA types expressed by the tissue
- These antibodies, pre-existing in a patient receiving a kidney allograft, can cause hyperacute renal graft rejection
  - Studies in late 1960s, Kissmeyer-Nielson, Terasaki
- Complement-dependent cytotoxicity (CDC) crossmatch becomes standard of practice in donor selection
- The appearance of donor-specific HLA-directed antibodies after transplant can lead to reduced graft survival

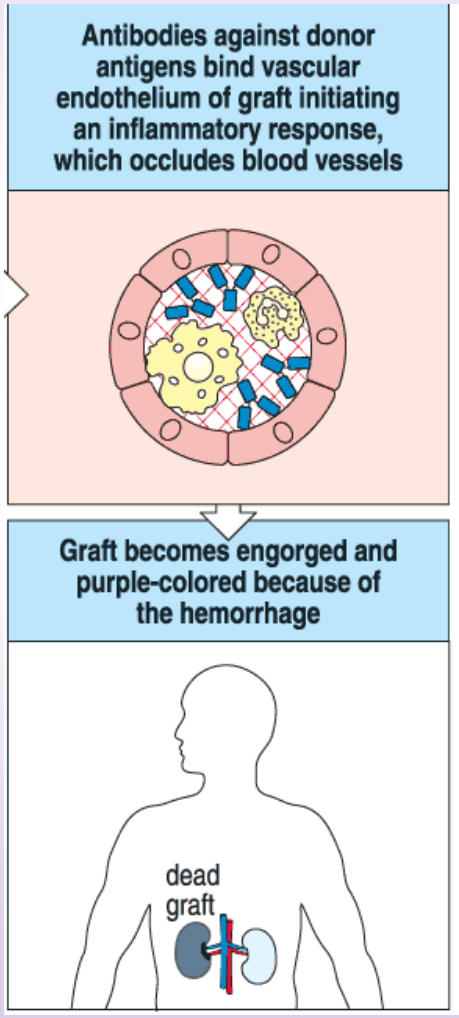


Fig 13.27 © 2001 Garland Science

Terasaki, *Transplantation*, 2012, 93:751

Impact of Early Research on Studies of Immune System / Transplantation as Model

- **MHC molecules present antigenic peptides to T lymphocytes, stimulating the adaptive immune response**
  - **Susceptibility/resistance to infectious disease, autoimmunity; drug sensitivity**
  - **Vaccine design**
  - **Immune escape of malignant cells**
- **Extensive genetic variation observed in MHC**
  - **Population biology; evolution including natural selection**
- **Ability to propagate transplanted cells/tissues in hosts in controlled environment**
  - **Transplantation as therapy, cellular therapies, pregnancy**
  - **Research models**

Tasmanian devil; facial tumor disease, a contagious cancer, is decimating this species

Ian Waldie/Getty Images, National Geographic

Forming the Histocompatibility Community

- 1st organized in 1964 by Amos at Duke University
- Opportunity to share reagents and unpublished data, develop new methods and standardize them, standardize nomenclature, disseminate knowledge world-wide
- 16 workshops with some highlights listed below
  - 1964 microcytotoxicity assay
  - 1965 cell panel, computer for analysis
  - 1970 eleven HL-A specificities
  - 1972 world-wide populations
  - 1975 class II defined as HLA-D
  - 1987 DNA typing
- 2017 17th Workshop
- 2022 18th Workshop (www.ihiw18.org)

http://www.ihwg.org/about/history.html

SEOPF to UNOS

1968	The Southeast Organ Procurement Foundation (SEOPF) is formed as a membership and scientific organization for transplant professionals.
1977	SEOPF implements the first computer-based organ matching system, dubbed the "United Network for Organ Sharing."
1982	SEOPF establishes the Kidney Center, the predecessor of the UNOS Organ Center, for round-the-clock assistance in placing donated organs.
1983	First successful single-lung transplant performed. Cyclosporine, the first of a number of drugs that effectively treat organ rejection by suppressing the human immune system, introduced.
1984	National Organ Transplant Act (NOTA) passed. United Network for Organ Sharing (UNOS) separates from SEOPF and is incorporated as a non-profit member organization.
2004	SEOPF becomes American Foundation for Donation and Transplantaion (AFDT)

Summary of History

- Beginning in early 1900s, the laws of transplantation were described
- Immunogenetic studies elucidating murine H-2 system began in 1930s
- For many years, the mouse was major model for MHC because of ability to address genetic diversity using inbred and congenic strains
- In 1950s, sensitization of humans to foreign tissue was observed; alloantibodies provide a tool to identify HLA types
- Research and collaboration through international workshops provided tools and standardization
- Led to understanding of immune response in health and disease, served as “the” model for genetic diversity and impact of natural selection, provided tools and models

Mechanisms of Allorecognition

- High frequency of alloreactive T cells arises because pathogen-specific memory T cells, as well as naïve T cells, can respond to allo-MHC
- Plasticity in TCR – MHC – peptide interaction produces cross-reactivity and activation
- Three pathways of activation (direct, indirect, semi-direct) predominate at different stages of rejection
- Minor antigens are recognized as MHC-bound peptides
- Naïve B cell activation requires recognition of foreign MHC by cell-surface antibody and T cell help

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Declaration of Istanbul

- **Initiated in 2008 by the WHO**
  - **as a response to unethical behaviors associated with organ transplantation and specifically organ trafficking**
  - **to address the urgent and growing problems of organ sales, transplant tourism and trafficking in organ donors in the context of the global shortage of organs**
- **Doesn't compel compliance**
  - **hoped that the principles and the proposals it outlines will guide and inspire better practices in transplantation.**
- **Endorsement of the Declaration**
  - **has been sought amongst the many professional societies associated with transplantation medicine.**

The DECLARATION of ISTANBUL on ORGAN TRAFFICKING and TRANSPLANT TOURISM

The Istanbul Summit

158 representatives of scientific and medical bodies, government officials, social scientists, and ethicists from around the world

Istanbul (Turkey), April 30 - May 2, 2008

Organ Trafficking

- **Act**
  - **the recruitment, transport, transfer, harboring or receipt of living or deceased persons or their organs**
- **By means of**
  - **the threat or use of force or other forms of coercion, abduction, fraud, deception, abuse of power or of a position of vulnerability, or payments to a third party to achieve control of the donor**
- **For purpose of**
  - **exploitation by the removal of organs for transplantation**

DoI Definitions

- **Transplant commercialism**
  - a policy or practice in which an organ is treated as a commodity, including by being bought or sold or used for material gain.
- **Travel for transplantation**
  - the movement of organs, donors, recipients or transplant professionals across jurisdictional borders for transplantation purposes.
- **Transplant tourism**
  - involves organ trafficking and/or transplant commercialism, or
  - organs, professionals and transplant centers devoted to providing transplants to patients from outside a country or region, undermine the country's ability to provide transplant services for its own population

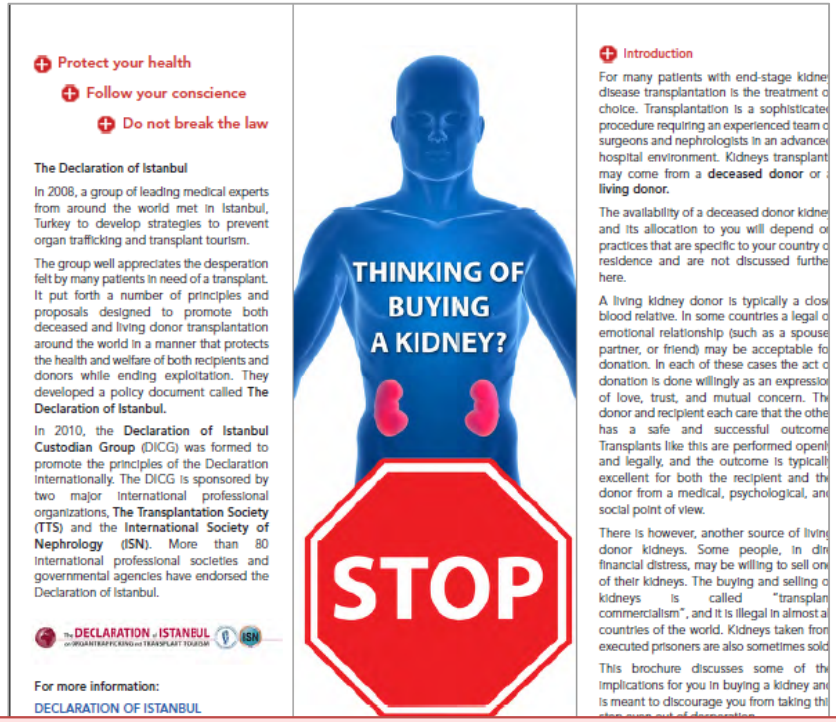
The DECLARATION of ISTANBUL on ORGAN TRAFFICKING and TRANSPLANT TOURISM

Materials to Help Combat Organ Trafficking and Transplant Tourism

Available on the website

- Full text of the Declaration of Istanbul
- Some proposals on how to promote the ethical practice of donation and transplantation
- Latest information on organ trafficking
- Patient Information/Brochure translated into many languages

Patient Information brochure



Protect your health

Follow your conscience

Do not break the law

The Declaration of Istanbul

In 2008, a group of leading medical experts from around the world met in Istanbul, Turkey to develop strategies to prevent organ trafficking and transplant tourism.

The group well appreciates the desperation felt by many patients in need of a transplant. It put forth a number of principles and proposals designed to promote both deceased and living donor transplantation around the world in a manner that protects the health and welfare of both recipients and donors while ending exploitation. They developed a policy document called **The Declaration of Istanbul.**

In 2010, the **Declaration of Istanbul Custodian Group** (DICG) was formed to promote the principles of the Declaration internationally. The DICG is sponsored by two major international professional organizations, **The Transplantation Society (TTS)** and the **International Society of Nephrology (ISN).** More than 80 international professional societies and governmental agencies have endorsed the Declaration of Istanbul.

For more information:

DECLARATION OF ISTANBUL

THINKING OF BUYING A KIDNEY?

STOP

Introduction

For many patients with end-stage kidney disease transplantation is the treatment of choice. Transplantation is a sophisticated procedure requiring an experienced team of surgeons and nephrologists in an advanced hospital environment. Kidneys transplant may come from a **deceased donor** or a **living donor.**

The availability of a deceased donor kidney and its allocation to you will depend on practices that are specific to your country of residence and are not discussed further here.

A living kidney donor is typically a close blood relative. In some countries a legal or emotional relationship (such as a spouse, partner, or friend) may be acceptable for donation. In each of these cases the act of donation is done willingly as an expression of love, trust, and mutual concern. The donor and recipient each care that the other has a safe and successful outcome. Transplants like this are performed openly and legally, and the outcome is typically excellent for both the recipient and the donor from a medical, psychological, and social point of view.

There is however, another source of living donor kidneys. Some people, in dire financial distress, may be willing to sell one of their kidneys. The buying and selling of kidneys is called "transplant commercialism", and it is illegal in almost all countries of the world. Kidneys taken from executed prisoners are also sometimes sold.

This brochure discusses some of the implications for you in buying a kidney and is meant to discourage you from taking this

www.declarationofistanbul.org

The DECLARATION of ISTANBUL on ORGAN TRAFFICKING and TRANSPLANT TOURISM

Home > About > List of Endorsing Organizations

ENDORSING ORGANIZATIONS

THE SPONSORING ORGANIZATIONS

The Transplantation Society

International Society of Nephrology
Advancing Nephrology Around the World

ENDORSING ORGANIZATIONS

(click on logos for website links)

Algerian Society of Nephrology, Dialysis and Transplantation

American Society for Histocompatibility & Immunogenetics (ASHI)

The DECLARATION of ISTANBUL on ORGAN TRAFFICKING and TRANSPLANT TOURISM

May 28, 2017

Dr Michael Gautreaux
President, American Society for Histocompatibility and Immunogenetics

Dear Dr Gautreaux,

As the Co-Chairs of the Executive Committee of the Board of Councillors of the **Declaration of Istanbul Custodian Group (DICG)**, we are writing to thank the American Society for Histocompatibility and Immunogenetics (ASHI) for endorsing the Declaration of Istanbul on Organ Trafficking and Transplant Tourism (DoI). On the following page, we would also like to share with you some information about the role that endorsing organizations can play in supporting the DoI and DICG activities.

In order to stay up-to-date with DICG activities and news relating to organ trafficking and transplant tourism around the world, ASHI can follow the DICG Facebook page or Twitter account. The DICG sends a quarterly newsletter via email to members and representatives of the endorsing organizations. This will be sent to the current President of ASHI, or a designated representative if you prefer. We ask that you add the address media@declarationofistanbul.org to your list of safe senders, to facilitate communications from our media account.

General information about the DICG's mission and current Board can be found on our website: www.declarationofistanbul.org. We will be delighted to address any particular questions that you or the members of ASHI may have. Please don't hesitate to get in touch at any time to discuss opportunities for collaboration. We look forward to working with you and the ASHI leadership and members in the coming years.

Warm regards,

Elmi Muller Dominique Martin

Co-chairs, DICG Executive Committee

Organizations which endorse the Declaration…

- require that speakers at scientific and educational meetings on clinical organ transplantation disclose whether the clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul.

- have an established mechanism for determining the appropriateness of accepting presentations on clinical organ transplantation based on the disclosure of a consistency with the Principles of the Declaration of Istanbul.

- establish mechanisms to promote, implement and uphold the Declaration (for example, through ethics committee activity, awards and membership criteria).

What can the lab personnel do?

- **Raise with transplant administrators if a patient appears to have participated in transplant tourism or some other form of organ trafficking.**

- **Understand that obstacles and disincentives to organ donation (living and deceased donor).**
  - **Vote!**

- **Share information, expertise and technology with labs in countries seeking to improve organ donation efforts.**

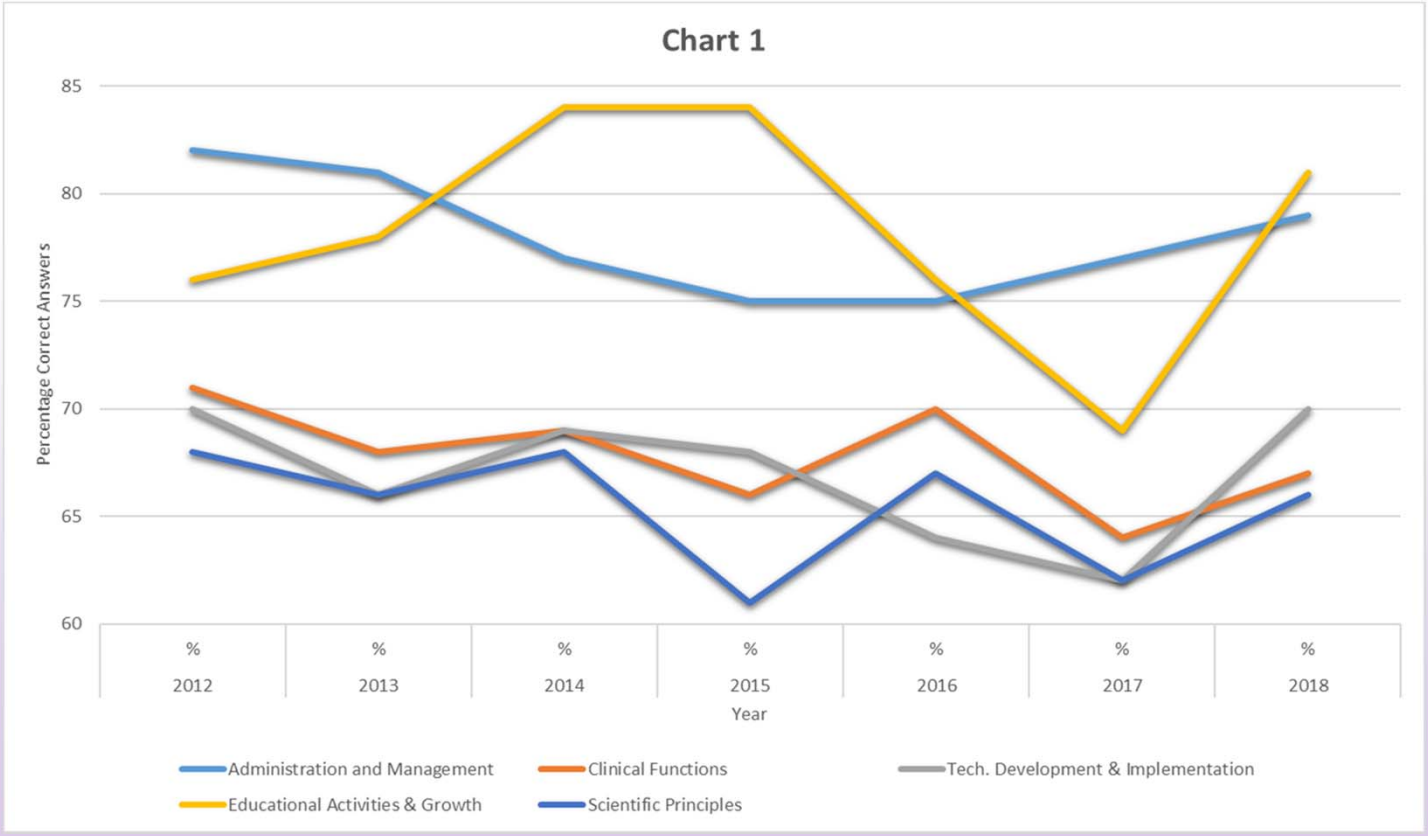
Overview of Transplant Immunology

- Innate and Adaptive Immunity
- Transplantation
- Brief History of Field
- Immune Response
- Declaration of Istanbul
- Wrap Up

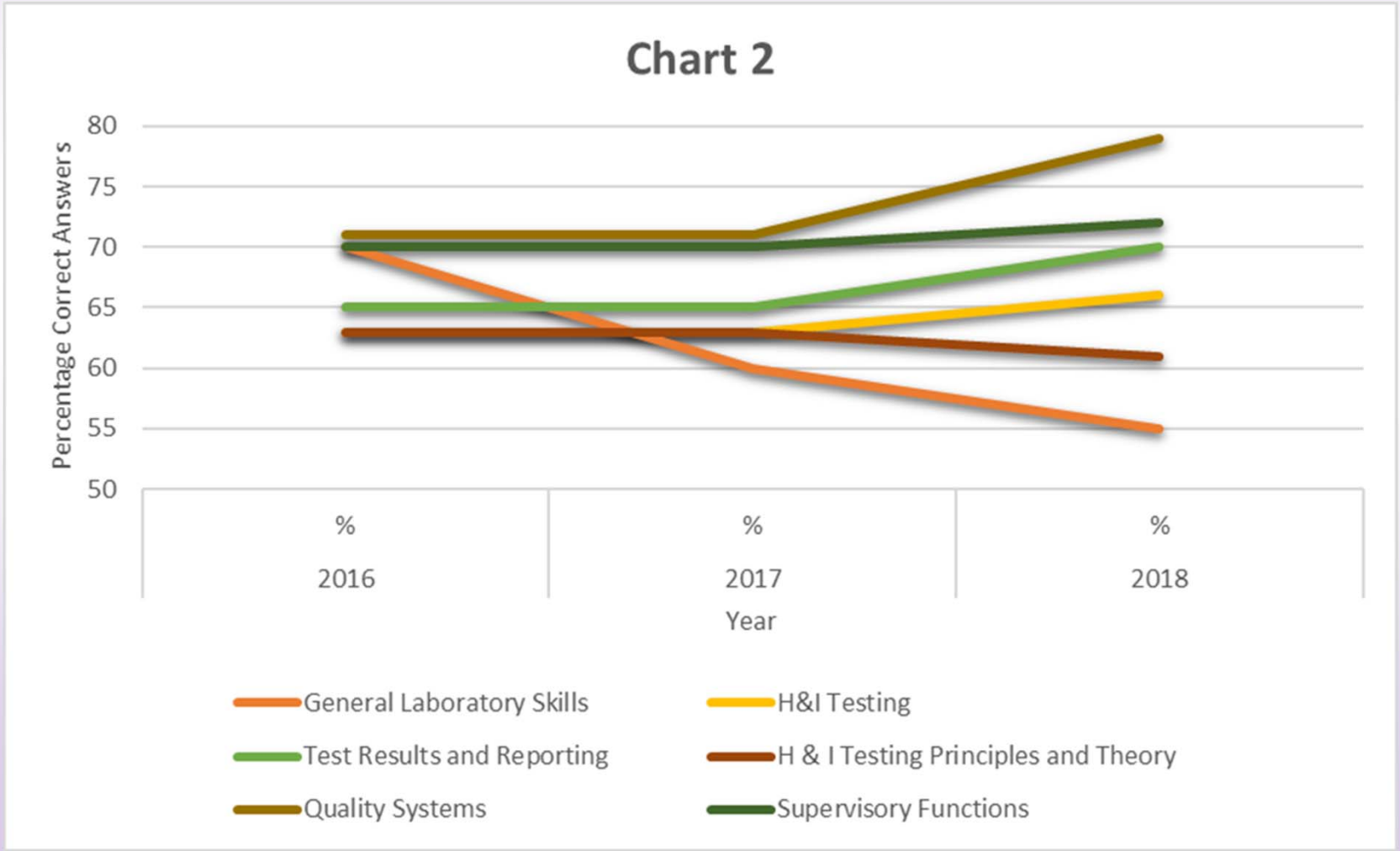
Soapbox Time!

Why Do We Cover So Much *Basic* Science?

- **2016 Specialists' Course Evaluation**
  - **[The Basic Immunology Lectures are] "too simplified for director level.**



CHS Examination



Why Do We Cover So Much Basic Science?

- **Knowledge of the science of immunogenetics is essential for proper performance and interpretation of the tests.**
- **We as a community need to understand the science so that we can explain why our testing is vitally important in the transplant field.**
  - **Younger people may want to be in this field**
  - **Patients & families are always looking for information**
  - **Policymakers are not sure what we do**
    - **"Don't they just stick some blood in a computer and get the result?"**
  - **"Regular" people don't know why what we do is important**
    - **Voters and taxpayers**
  - **Medical Professionals**

Thanks!


THANK YOU


Merci


Gracias


Hvala


Obrigado!


Ευχαριστώ


תודה


شكراً


Vielen
Dank


ขอบคุณ


Díky


Grazie


Bedankt