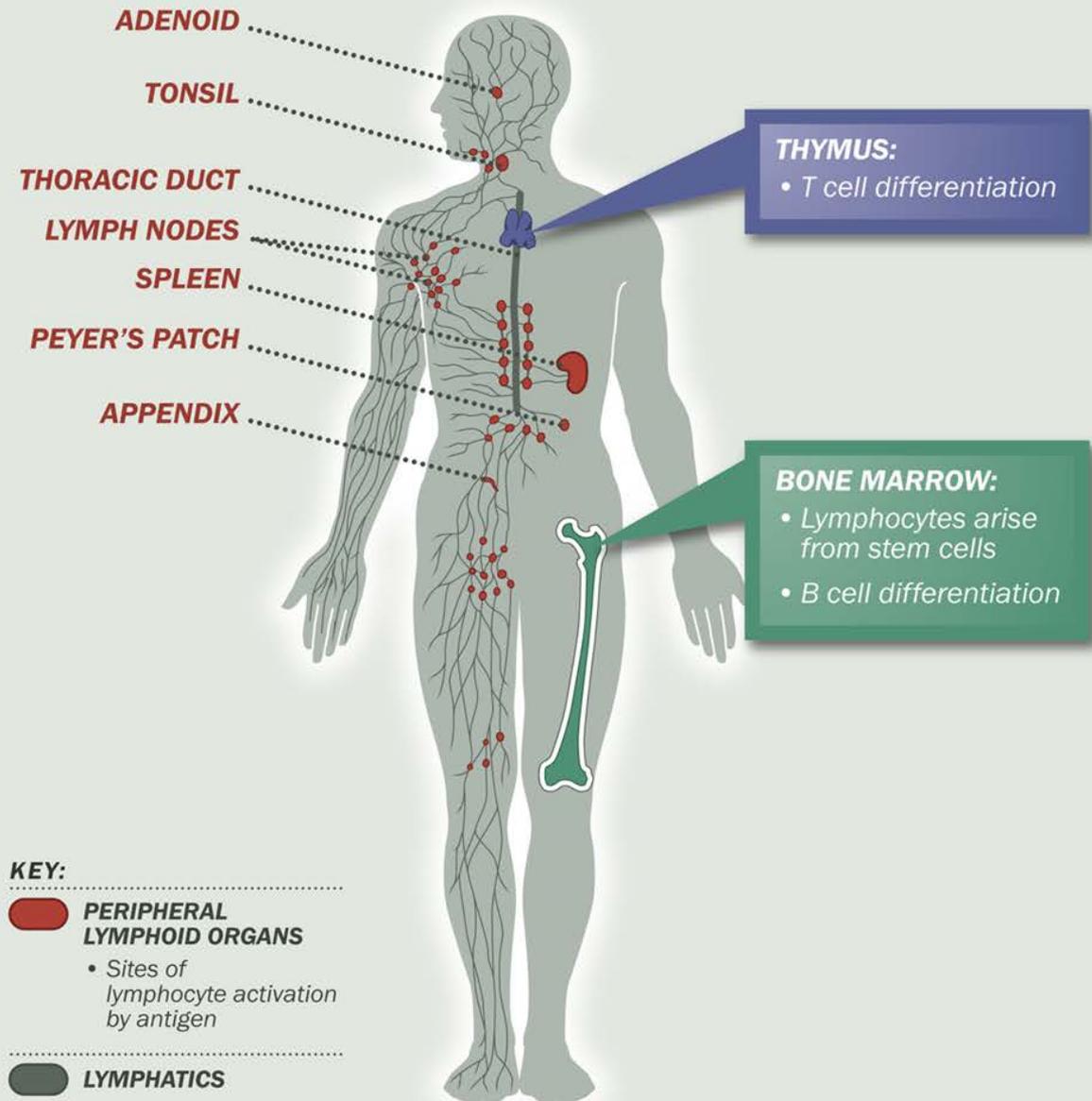
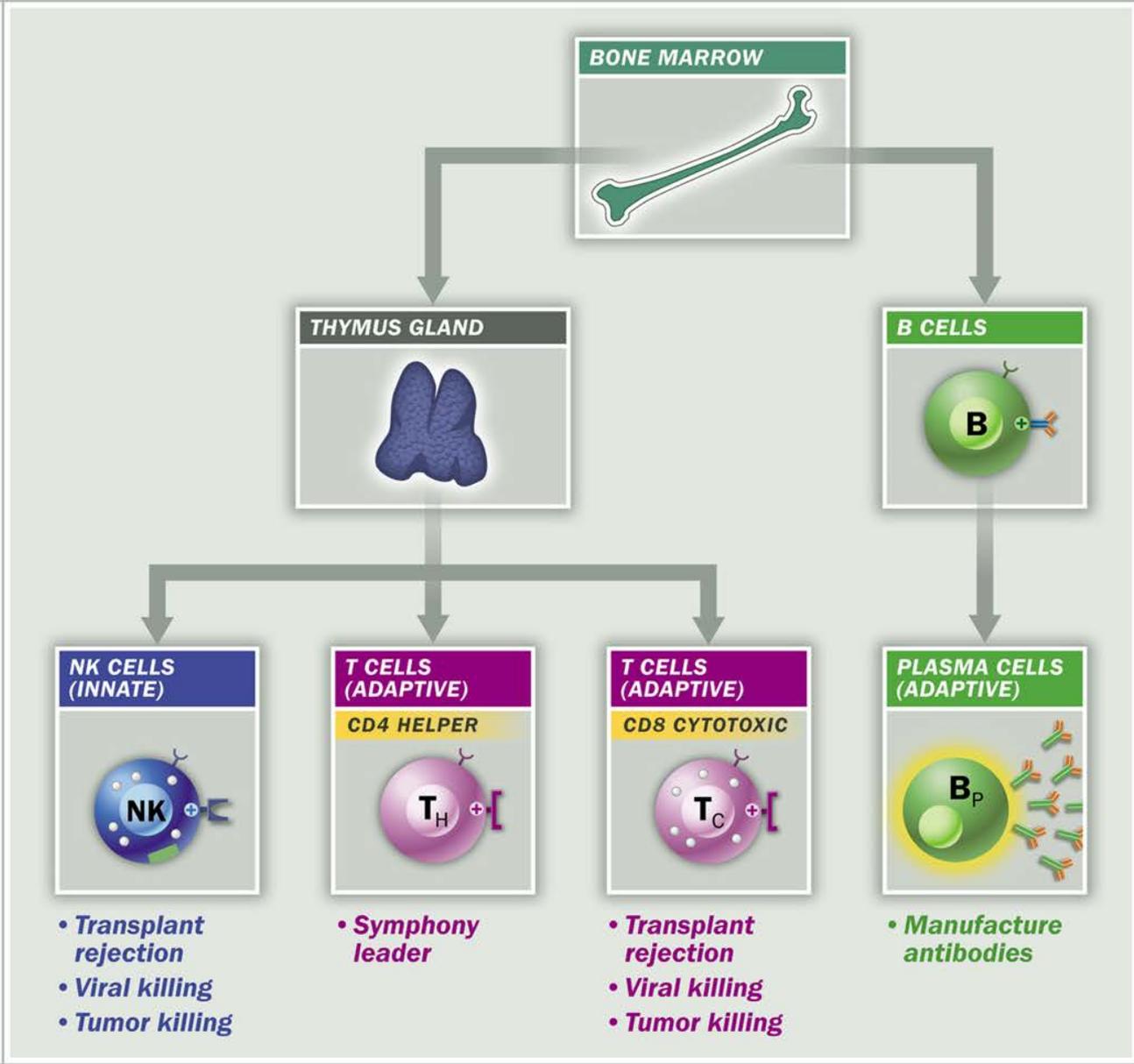


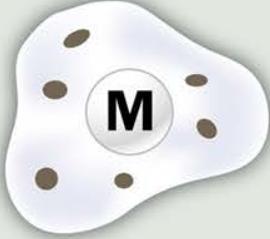
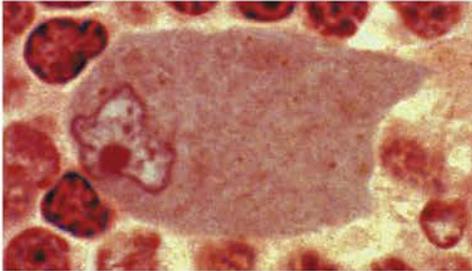
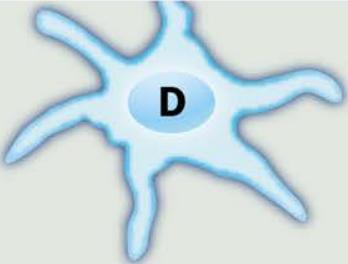
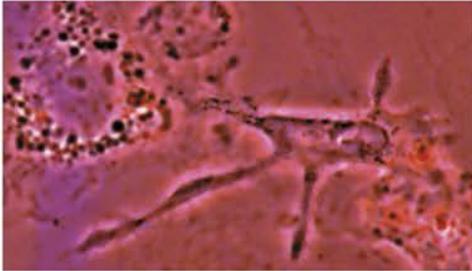
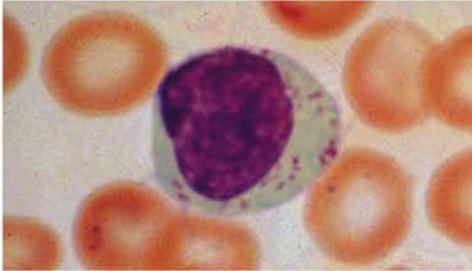
Distribution of Lymphoid Tissues in the Body



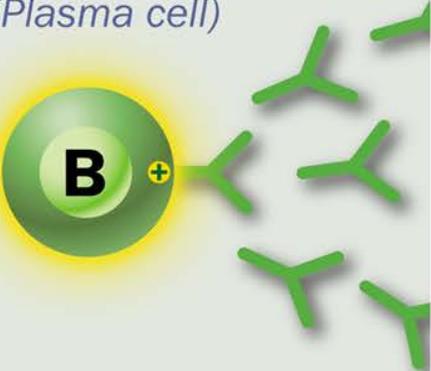
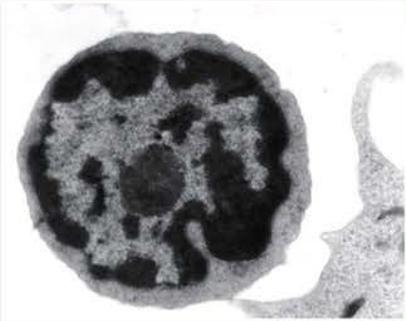
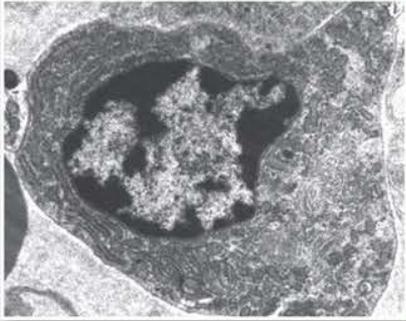
Differentiation of T cells and B cells



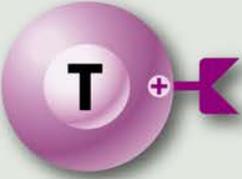
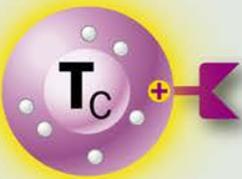
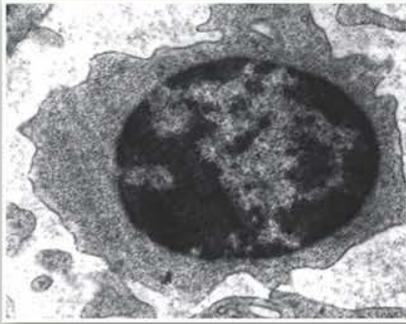
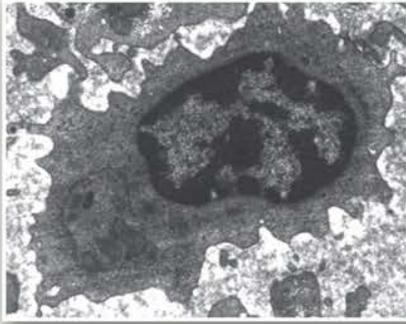
Cells of the Innate Immune System

MACROPHAGE	PHOTOMICROGRAPH	FUNCTION
		<ul style="list-style-type: none"> • Ingestion and elimination of foreign cells (Phagocytosis) • Antigen presentation • Secretion of TNF-α and IL-6
DENDRITIC CELL	PHOTOMICROGRAPH	FUNCTION
		<ul style="list-style-type: none"> • Antigen uptake in peripheral sites • Antigen presentation • Secretion of IFN-γ
NATURAL KILLER (NK) CELL	PHOTOMICROGRAPH	FUNCTION
		<ul style="list-style-type: none"> • Release of lytic granules that kill virus infected cells • Secretion of IFN-γ

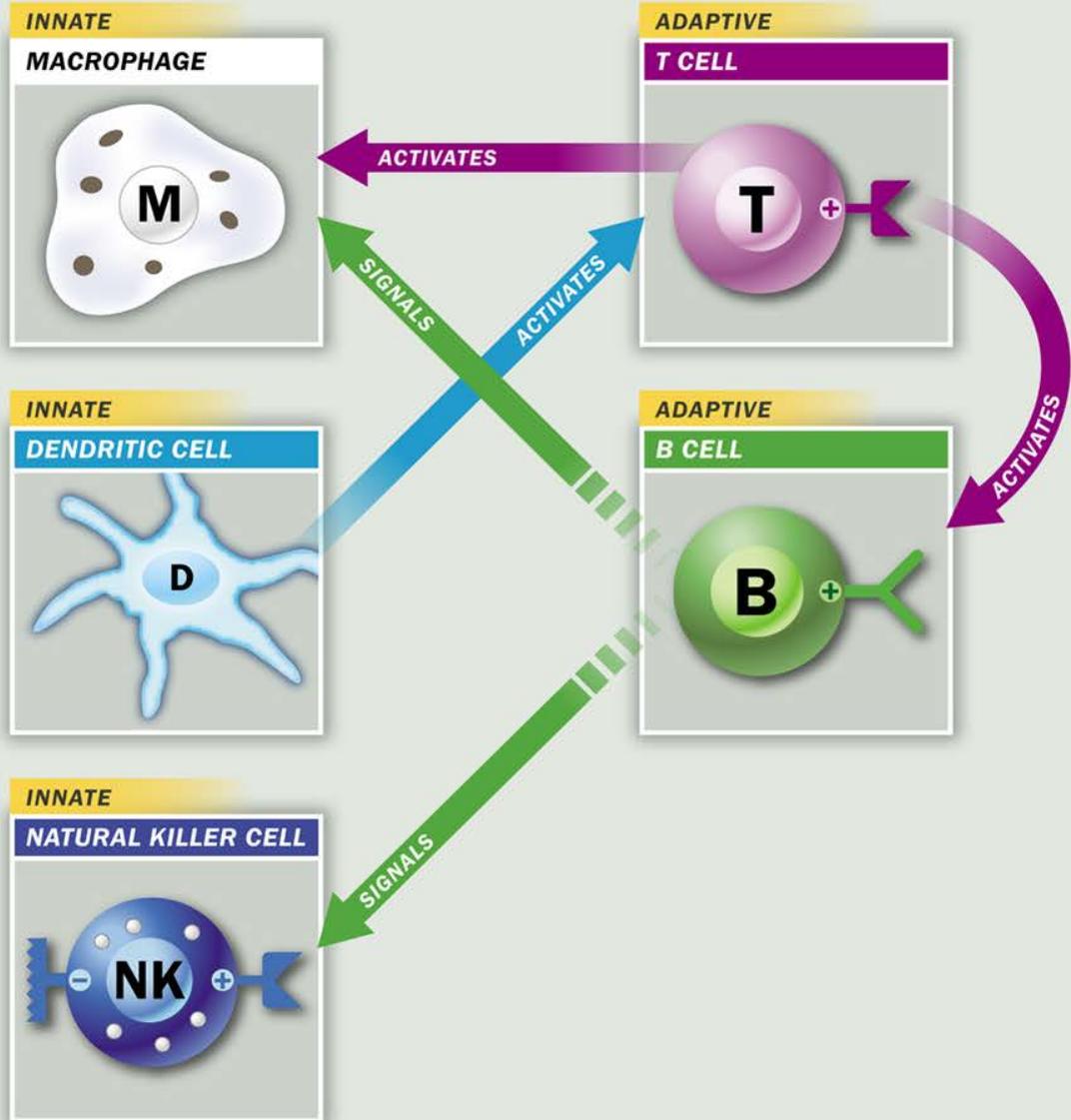
Cells of the Adaptive Immune System

B CELLS	TEM	FUNCTION
<p data-bbox="112 382 372 419">Resting B cell</p>  <p data-bbox="112 876 407 958">Activated B cell (Plasma cell)</p> 	<p data-bbox="604 382 1006 464"><i>Transmission Electron Micrograph</i></p>  <p data-bbox="604 876 1006 958"><i>Transmission Electron Micrograph</i></p> 	<ul data-bbox="1097 382 1798 1076" style="list-style-type: none">• Recognize and bind to specific antigens• Produce antibodies that bind to foreign cells, forming an antigen/antibody complex• Antigen/antibody complex flags foreign cells for macrophage ingestion and destruction• Bound antibodies can also signal the complement system to destroy the foreign cell

Cells of the Adaptive Immune System

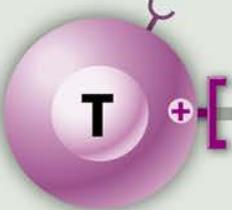
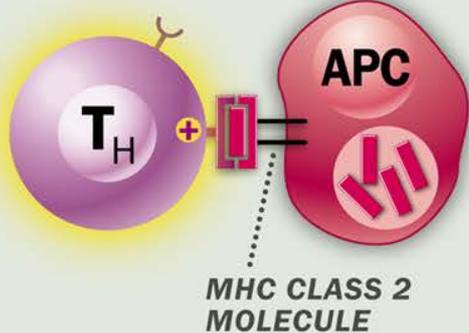
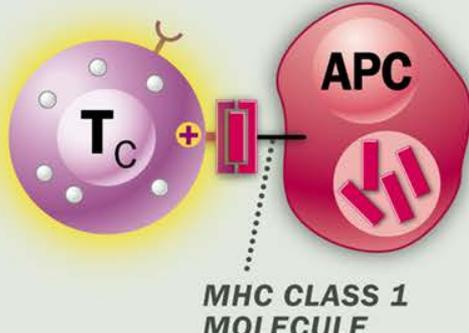
T CELLS	TEM	FUNCTION
<p data-bbox="112 382 363 421"><i>Resting T cell</i></p>  <p data-bbox="112 878 479 949"><i>Activated Cytotoxic T cell</i></p> 	<p data-bbox="602 382 1008 464"><i>Transmission Electron Micrograph</i></p>  <p data-bbox="602 878 1008 959"><i>Transmission Electron Micrograph</i></p> 	<ul data-bbox="1091 382 1825 1302" style="list-style-type: none">• T Cell Receptors (TCRs) recognize specific antigens• Activated T cells differentiate into three types of cells<ul style="list-style-type: none">– Cytotoxic T cells which seek out infected cells and release lytic granules that destroy foreign cells– Helper T cells that:<ul style="list-style-type: none">– activate B cells to mature into antibody producing plasma cells– activate macrophages and increase their cytotoxic activity– signal and activate additional cytotoxic T cells– Memory T cells that memorize the antigen so it can be dealt with quickly upon re-exposure

Interaction between the Innate and Adaptive Immune Responses



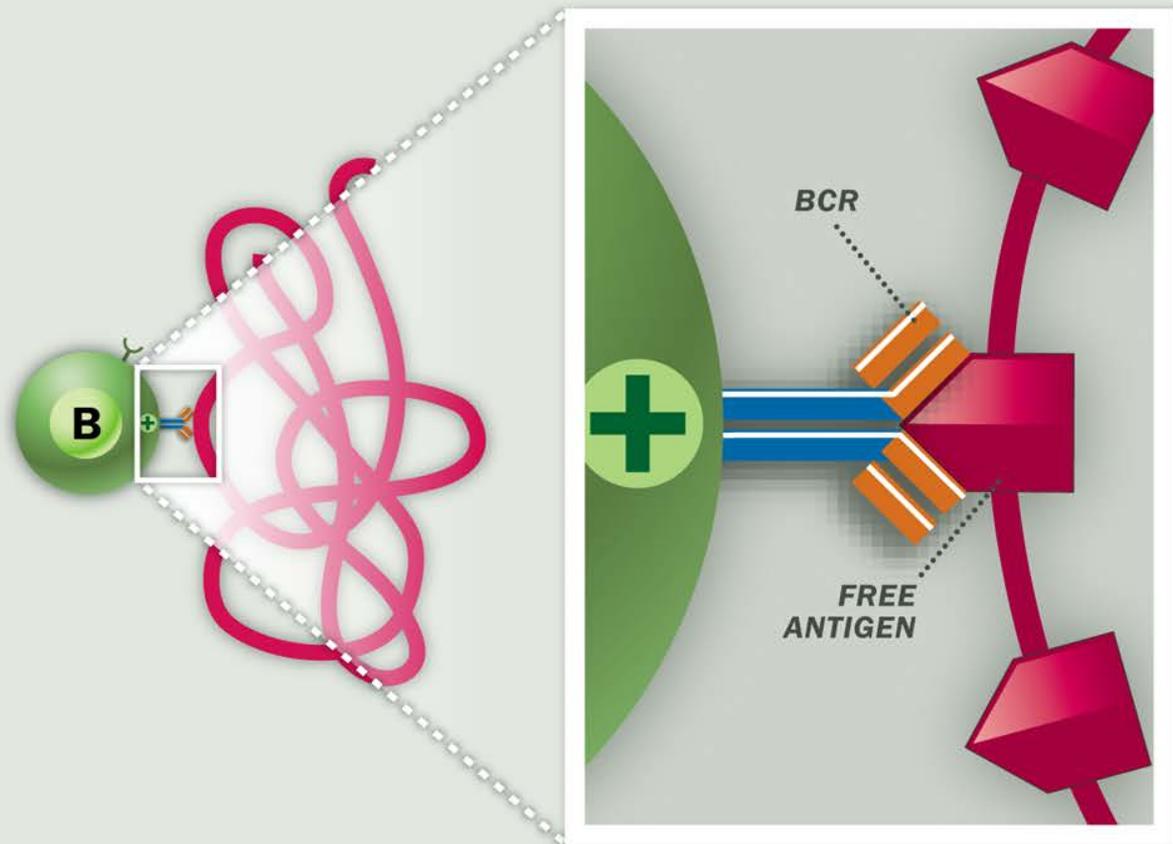
Cells of the Adaptive Immune System

CD4 Helper T cells and CD8 Cytotoxic T cells

T CELLS	EFFECTOR T CELLS	ROLE
<p><i>Naïve T cell</i></p> 	<p><i>CD4 Helper T cell</i></p>  <p><i>CD8 Cytotoxic T cell</i></p> 	<ul style="list-style-type: none">• Recognizes specific antigen fragment presented in association with an MHC Class 2 molecule on the surface of an APC.<hr/>• Recognizes specific antigen fragment presented in association with an MHC Class 1 molecule on the surface of an APC.

Antigen Recognition by B cells and T cells

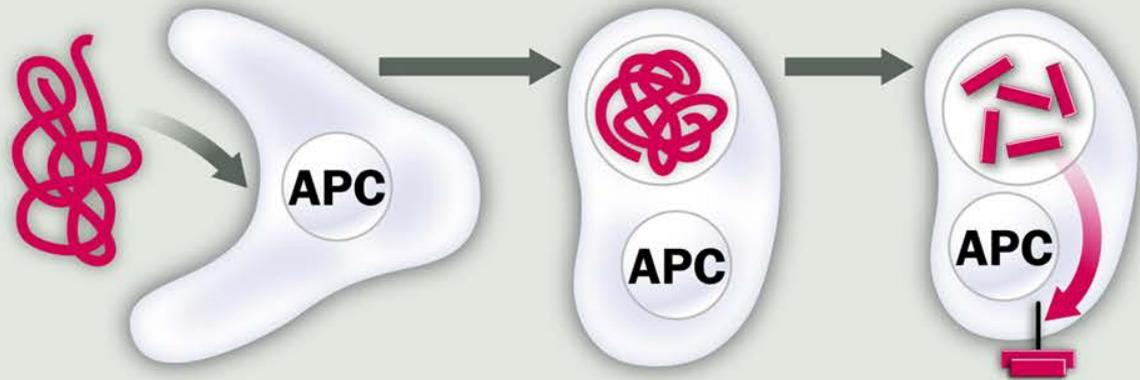
B cells **recognize the 3-dimensional structure** of free antigen **without the need for digestion (processing)** of the antigen.



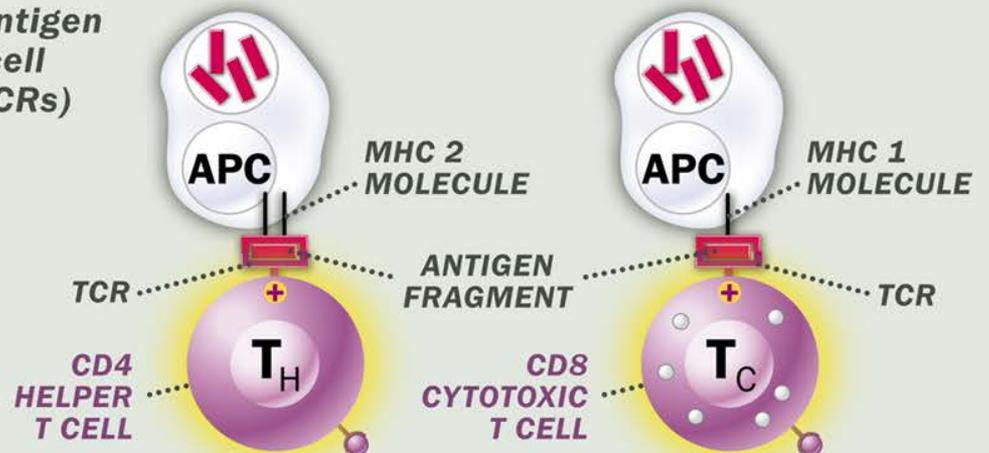
Antigen Recognition by B cells and T cells

T cells **recognize a linear piece of the antigen that has been digested (processed)** by the Antigen Presenting Cells (APCs) and presented on their surface in association with MHC molecules (MHC 1 and MHC 2).

Antigen is processed by APC:



Processed antigen activates T cell receptors (TCRs)



THE QUESTION:

How can the immune system of a mouse be removed so that the mouse can be used as a “petri dish” to conduct experiments that cannot ethically be conducted in humans?

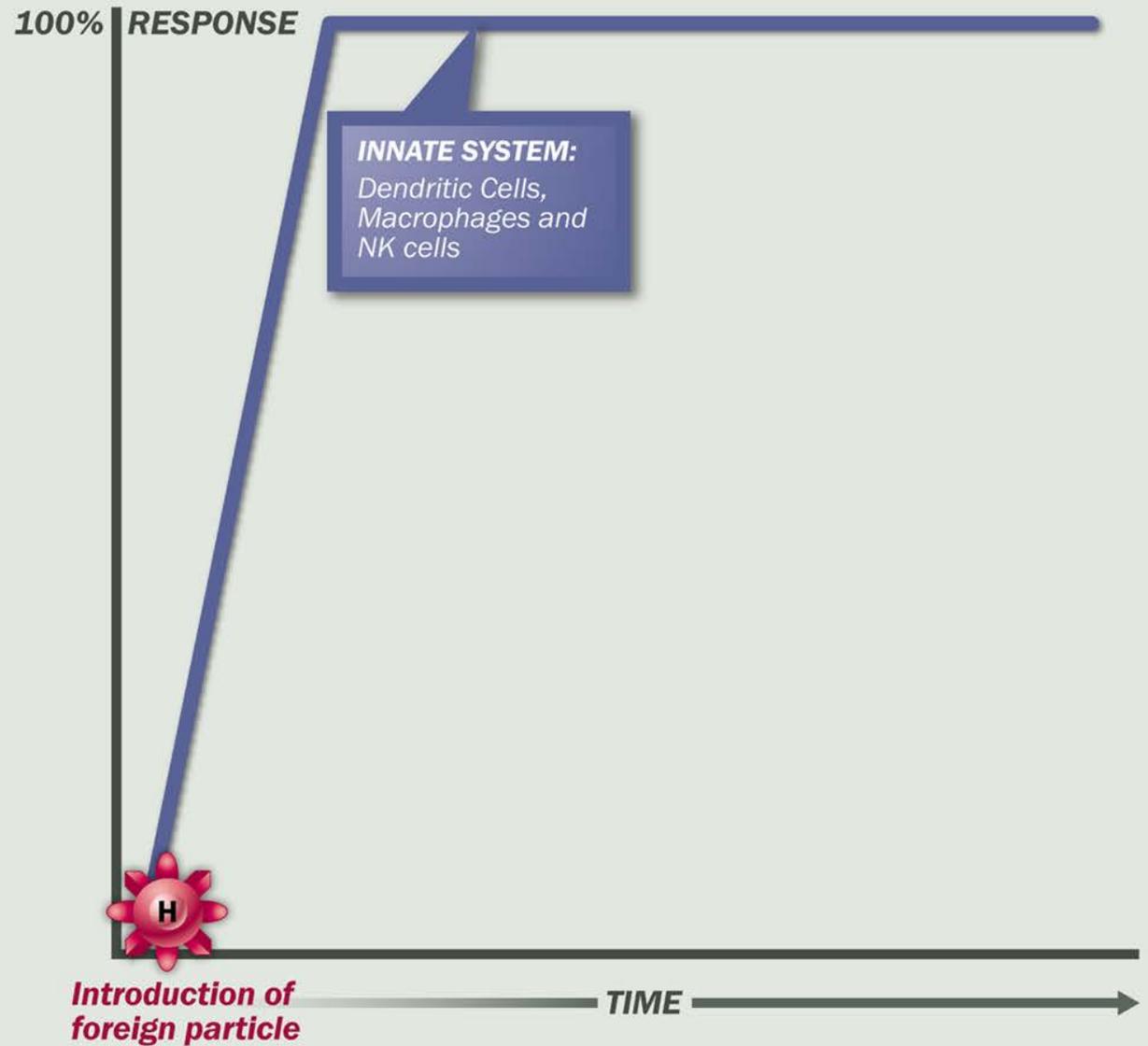
Examples of Human Immunodeficiencies That Affect Immune Cell Development and Function

WHAT IS THE CONSEQUENCE OF:	IMMUNODEFICIENCY
→ No T cells	SCID 1 Disease
→ No B cells	X-linked agammaglobulinemia
→ No T and B cells	SCID 2 Disease
→ No NK cells	STAT 5 Deficiency
→ No T, B and NK cells	SCID 3 Disease
→ No IFN-γ production or No IFN-γ receptors	Absence of macrophage killing

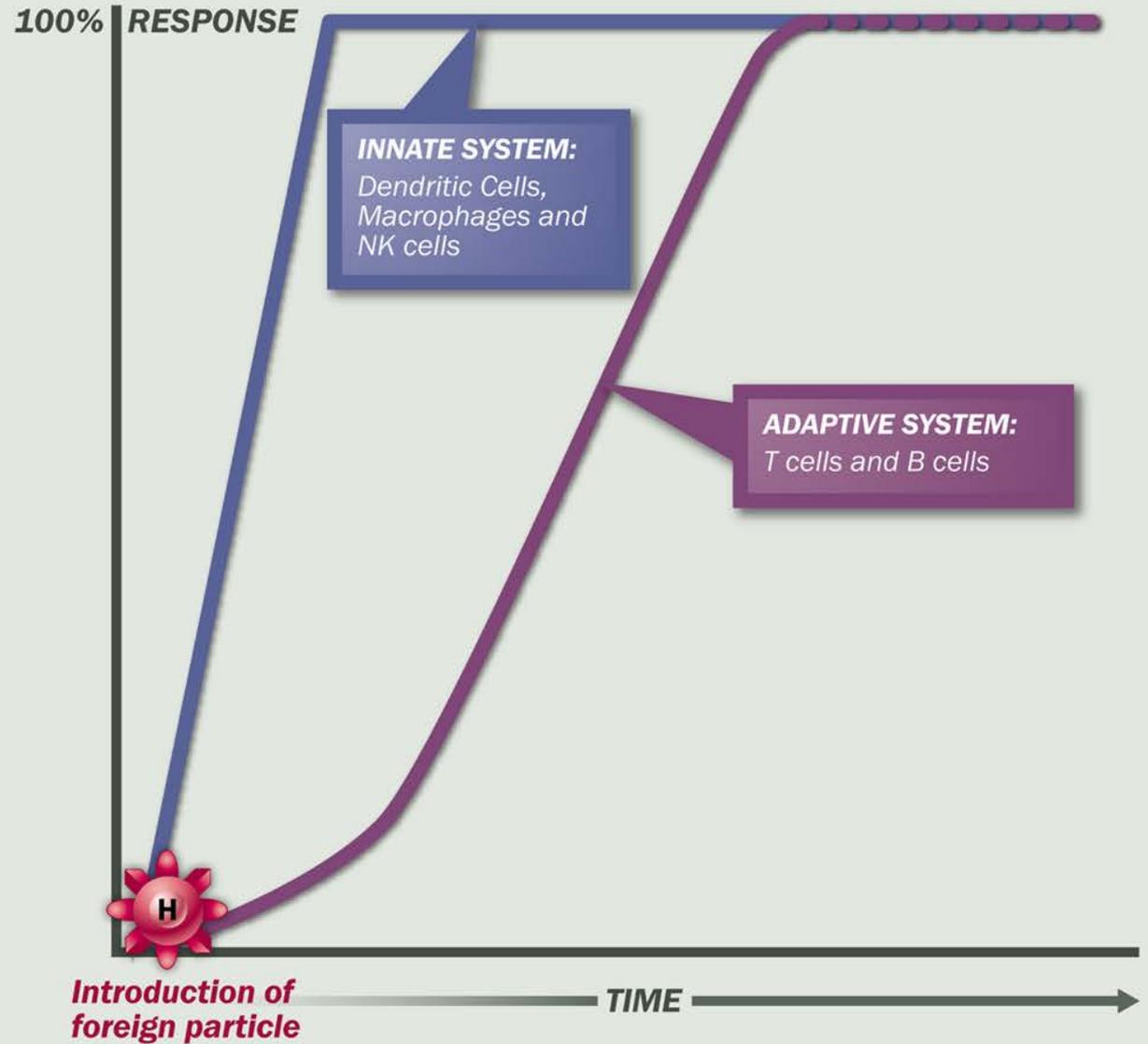
Immune System Overview

	INNATE IMMUNE SYSTEM	ADAPTIVE IMMUNE SYSTEM
<p>Receptors (Molecules expressed on the cell surface which perform a signaling function once triggered)</p>	<p>➔ Broad specificity:</p> <ul style="list-style-type: none"> - 1 receptor recognizes multiple molecules and/or organisms, i.e. bacteria and viral DNA 	<p>➔ Extremely narrow specificity:</p> <ul style="list-style-type: none"> - 1 receptor recognizes a single molecule of a foreign particle or cell
<p>Cells that Can Express the Receptor</p>	<p>➔ Static and limited in number</p> <p>➔ All cells:</p> <ul style="list-style-type: none"> - Immune (i.e. macrophages, dendritic and NK cells) and non-immune (i.e. skin, liver) 	<p>➔ Dynamic and nearly infinite in number</p> <p>➔ Only immune cells:</p> <ul style="list-style-type: none"> - T and B cells
<p>Frequency of Receptor-Expressing Cells</p>	<p>➔ High frequency:</p> <ul style="list-style-type: none"> - Up to 100% 	<p>➔ Very low frequency:</p> <ul style="list-style-type: none"> - 1 in 100,000
<p>Role</p>	<p>➔ First line of defense:</p> <ul style="list-style-type: none"> - Hours to a few days - Eliminates more than 90% of foreign particles 	<p>➔ Second line of defense:</p> <ul style="list-style-type: none"> - A week plus - Requires time for cell expansion and maturation

Kinetics of Innate Immune System Response Compared to Adaptive Immune System Response

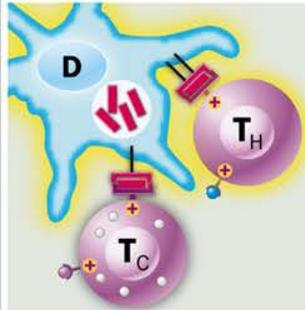


Kinetics of Innate Immune System Response Compared to Adaptive Immune System Response



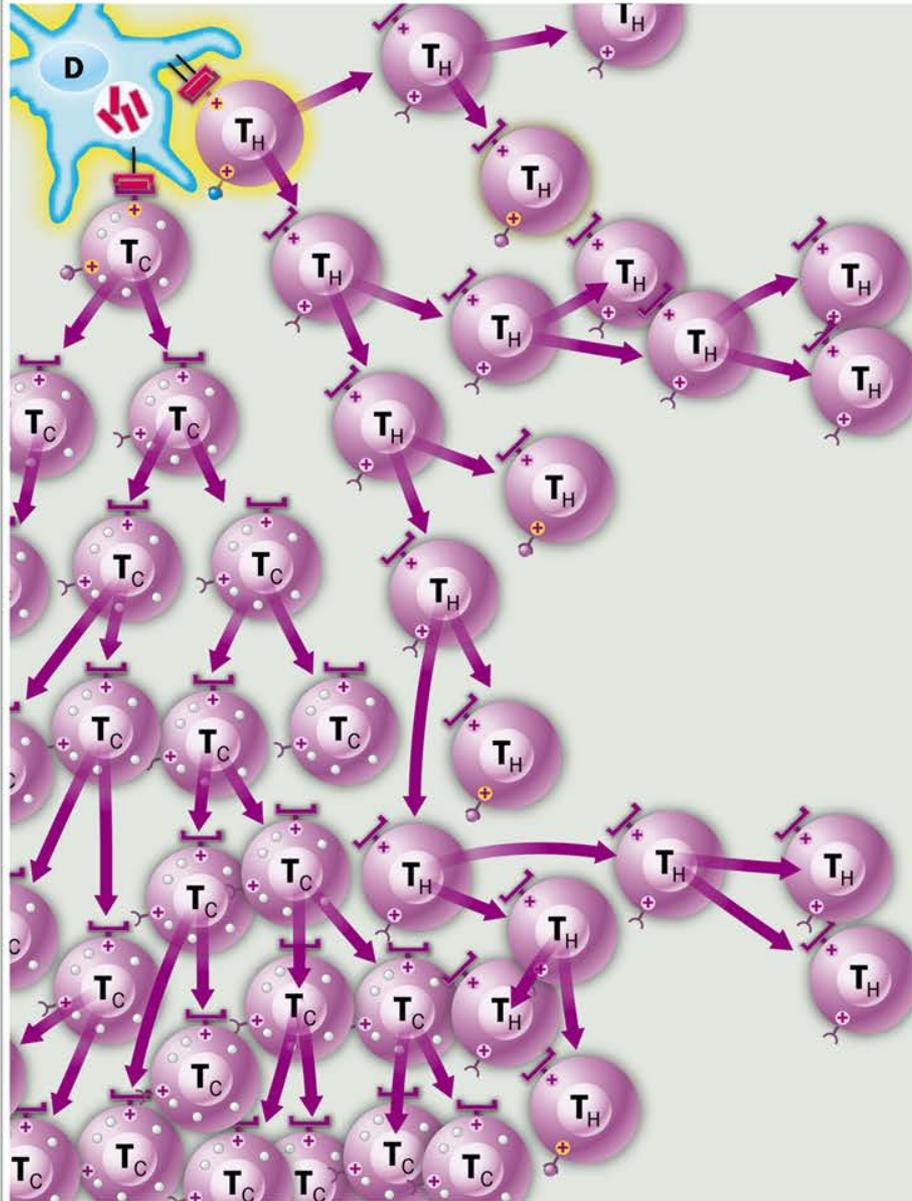
Expansion of T cells and B cells

The Adaptive
Immune Response
Occurs Later
Than the Innate
Immune Response
Because Adaptive
Cells Need Time
to Proliferate
and Mature



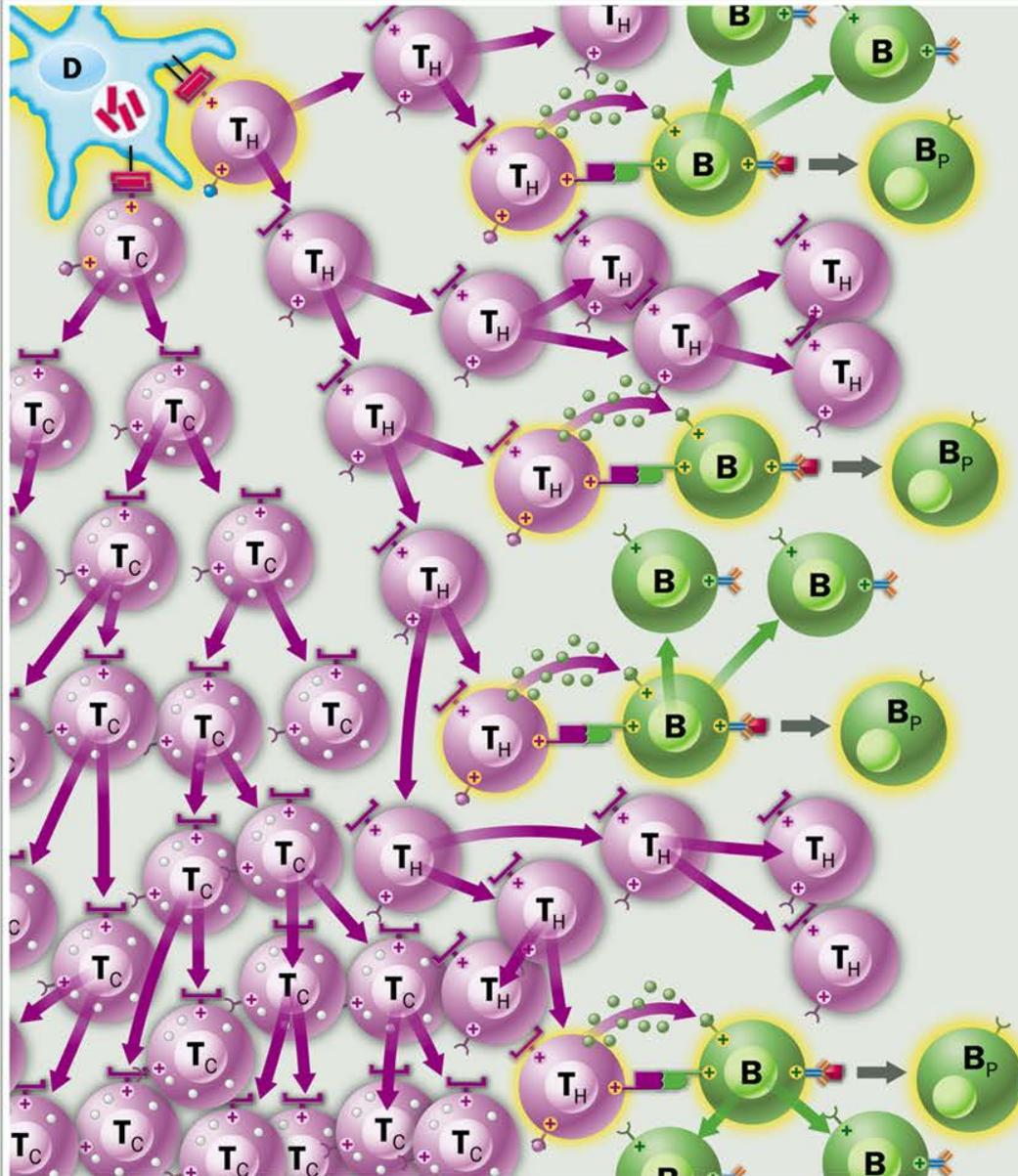
Expansion of T cells and B cells

Activated T cells
Differentiate
into Additional
Helper (CD4) and
Cytotoxic (CD8)
T cells



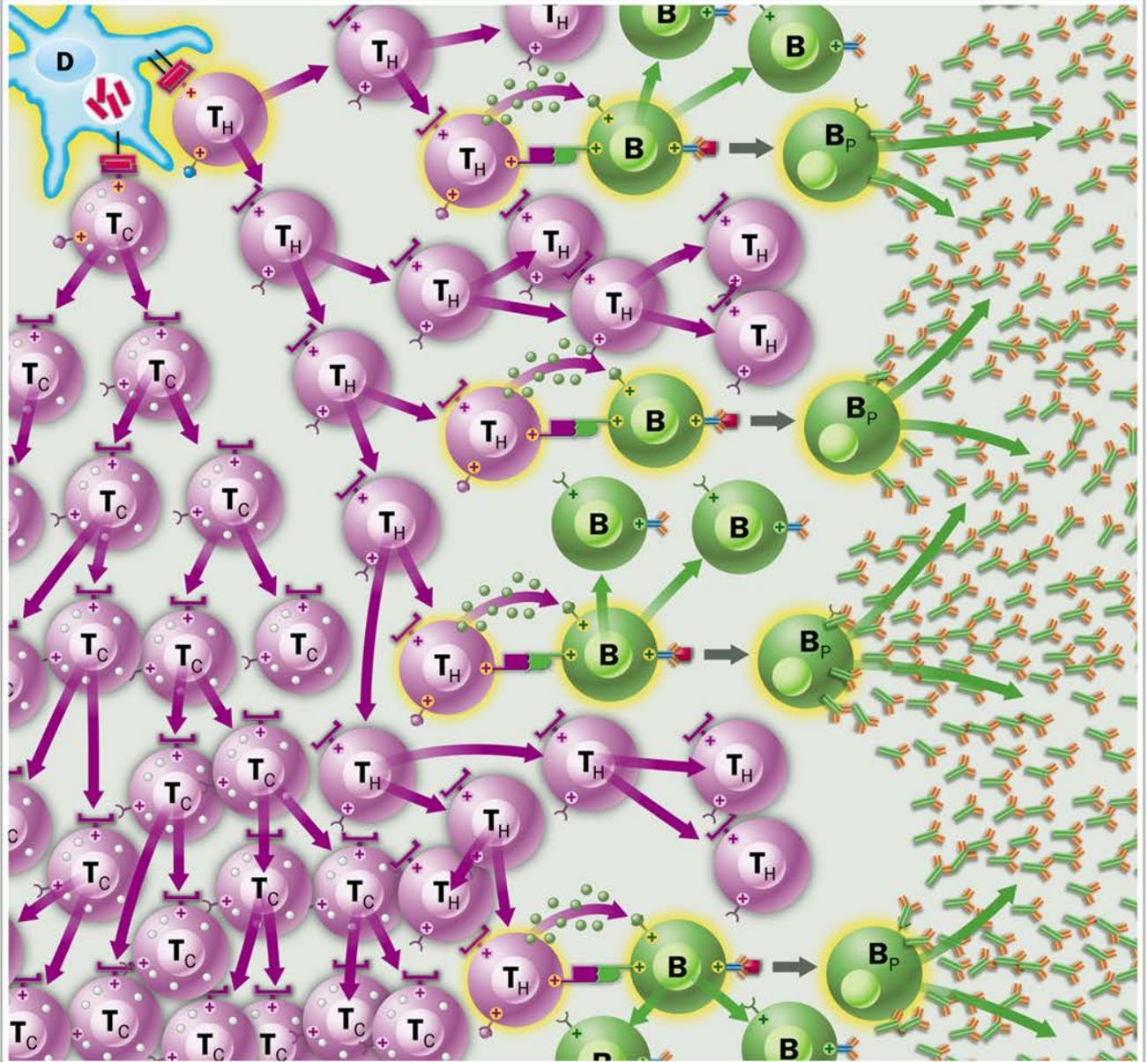
Expansion of T cells and B cells

CD4 Helper T cells
Activate B cells,
Causing them
to Proliferate
and Mature into
Antibody Producing
Plasma Cells



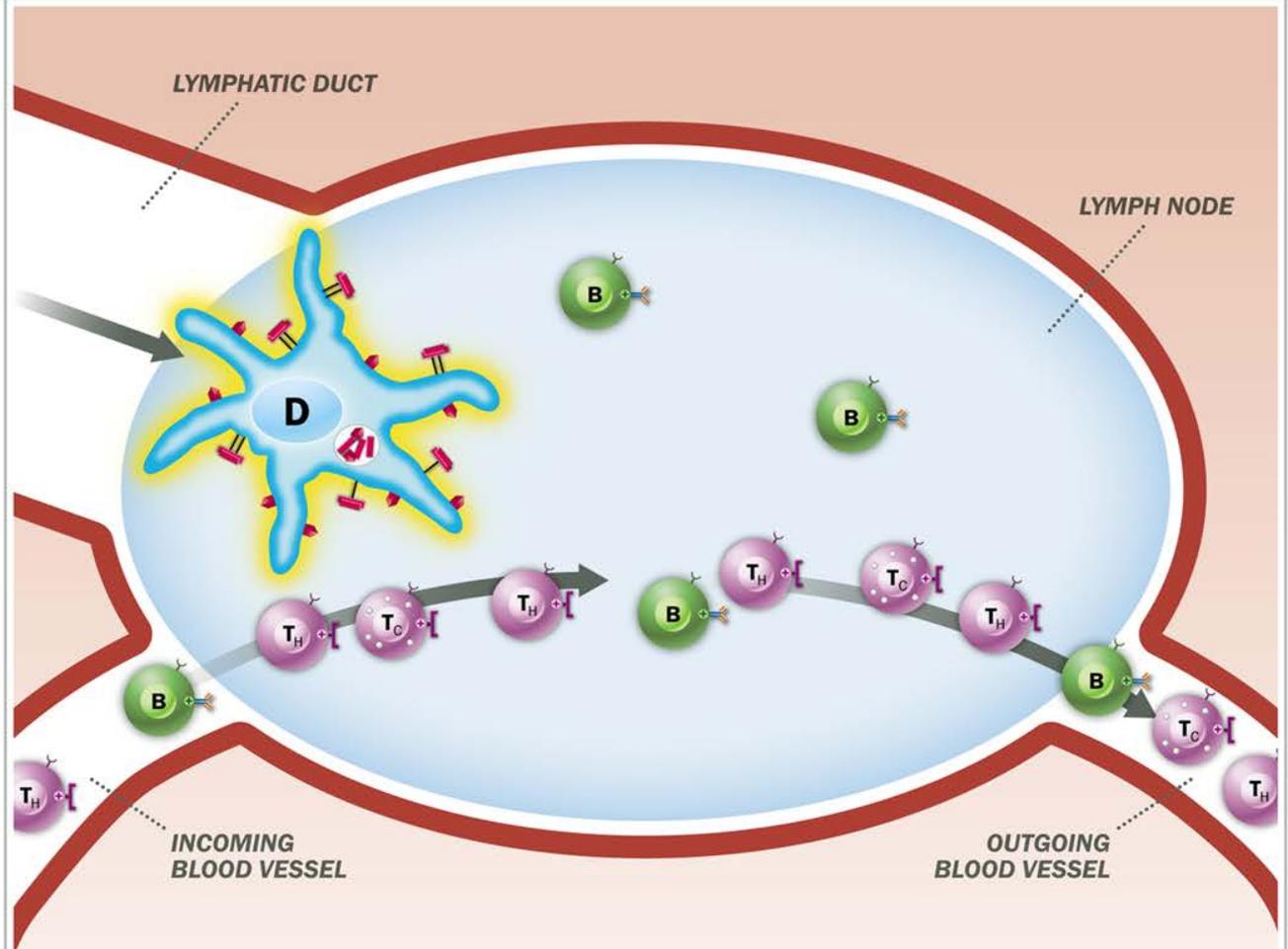
Expansion of T cells and B cells

CD4 Helper T cells
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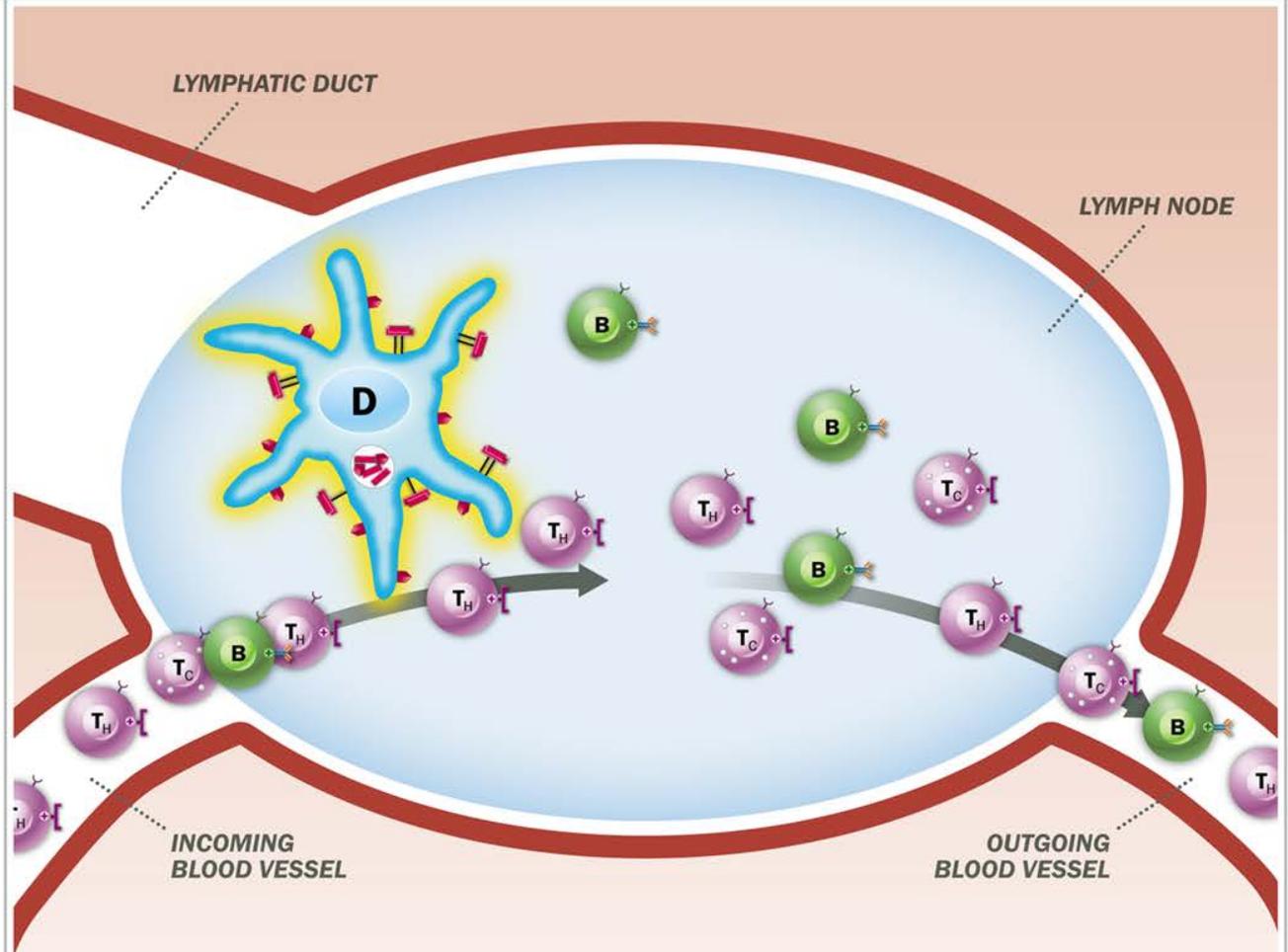
Expansion of T cells and B cells

The Dendritic Cell Enters the Lymphatic Vessel and Travels to the Lymph Node



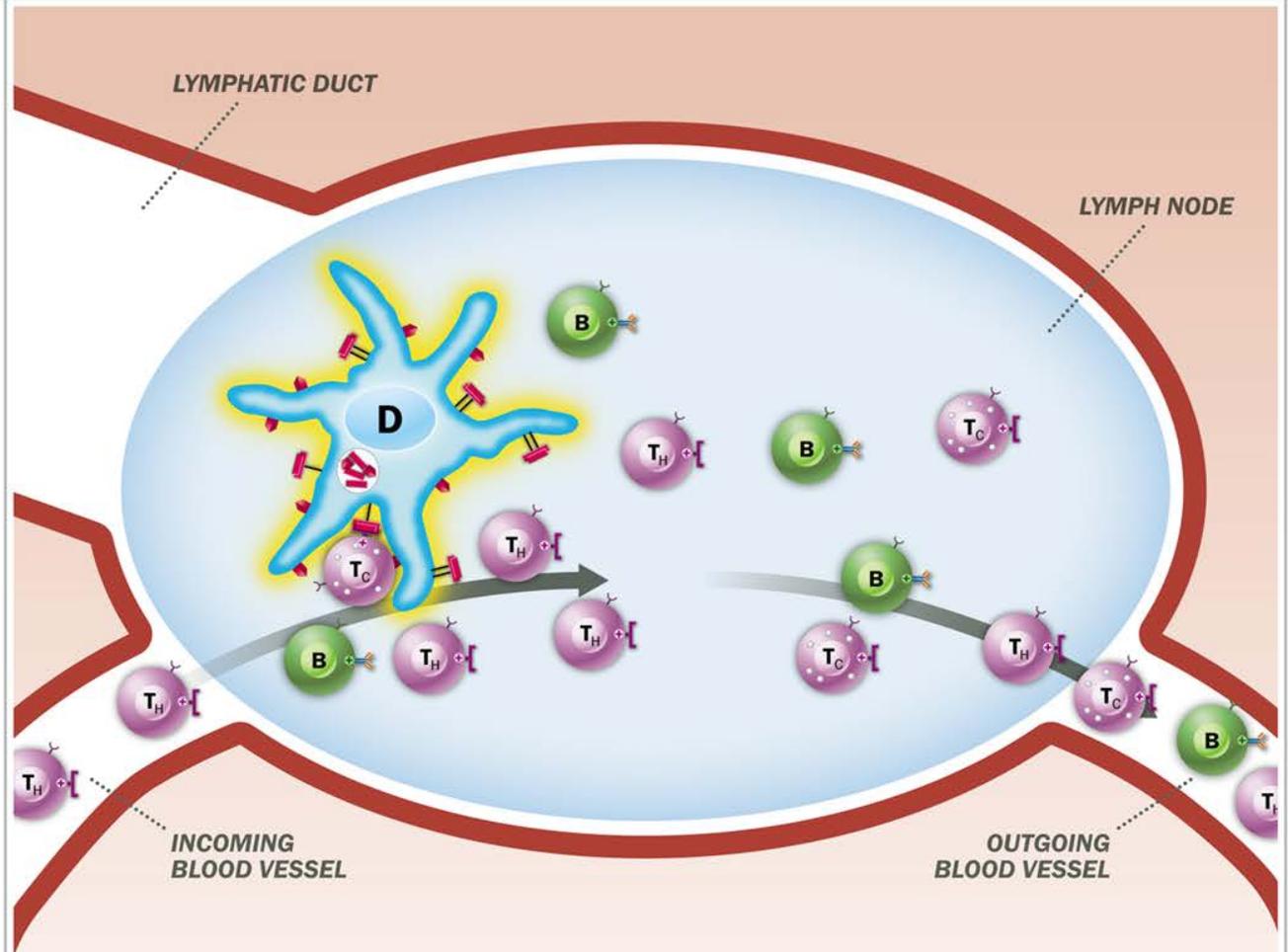
Expansion of T cells and B cells

T cells and B cells
Continuously
Circulate through
the Lymph Node
Searching for
Foreign Cells or
Particles



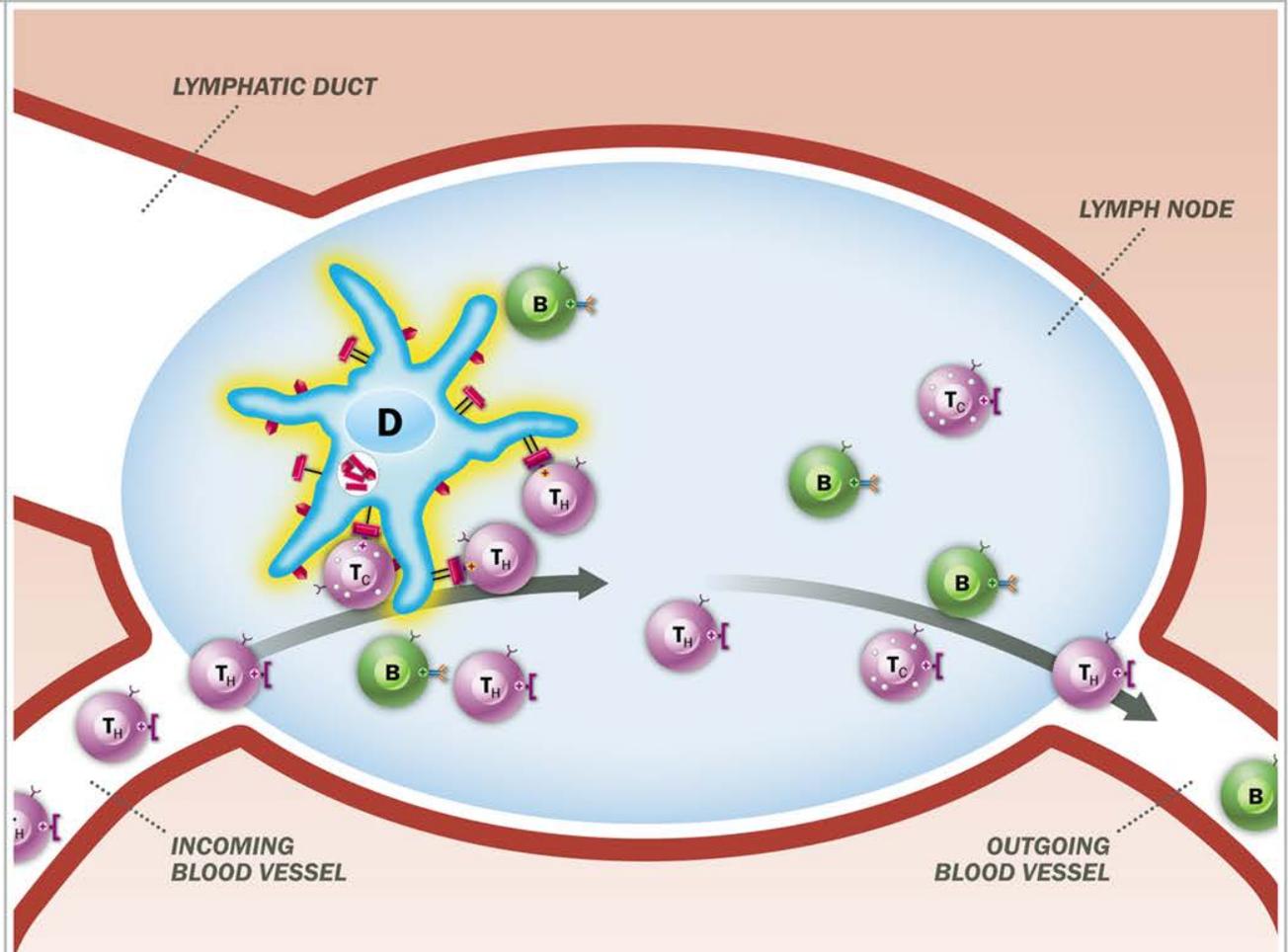
Expansion of T cells and B cells

If a T cell Recognizes an MHC Molecule Loaded with Antigen Specific to the T cell Receptor, It Will Stop and Interact with the Dendritic Cell



Expansion of T cells and B cells

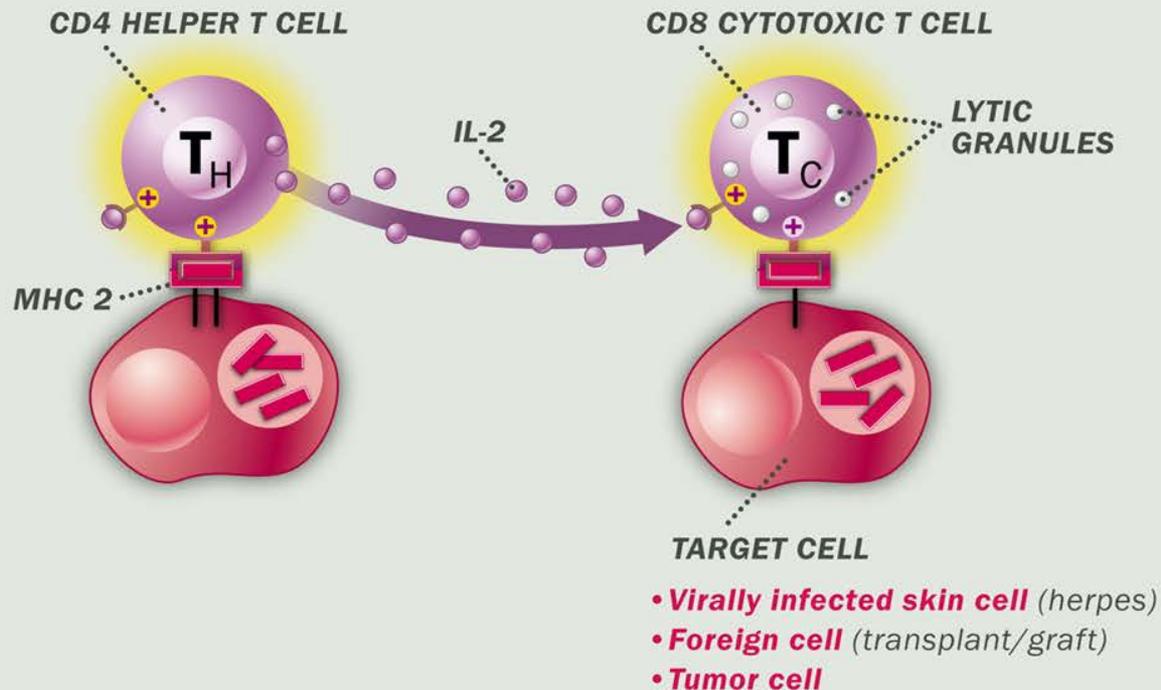
T cells Bind to the MHC Molecule Loaded with Antigen Fragment Specific to its T cell Receptor



Role of T cells

Antigen Fragment Presented on APC Activates T cell Receptor

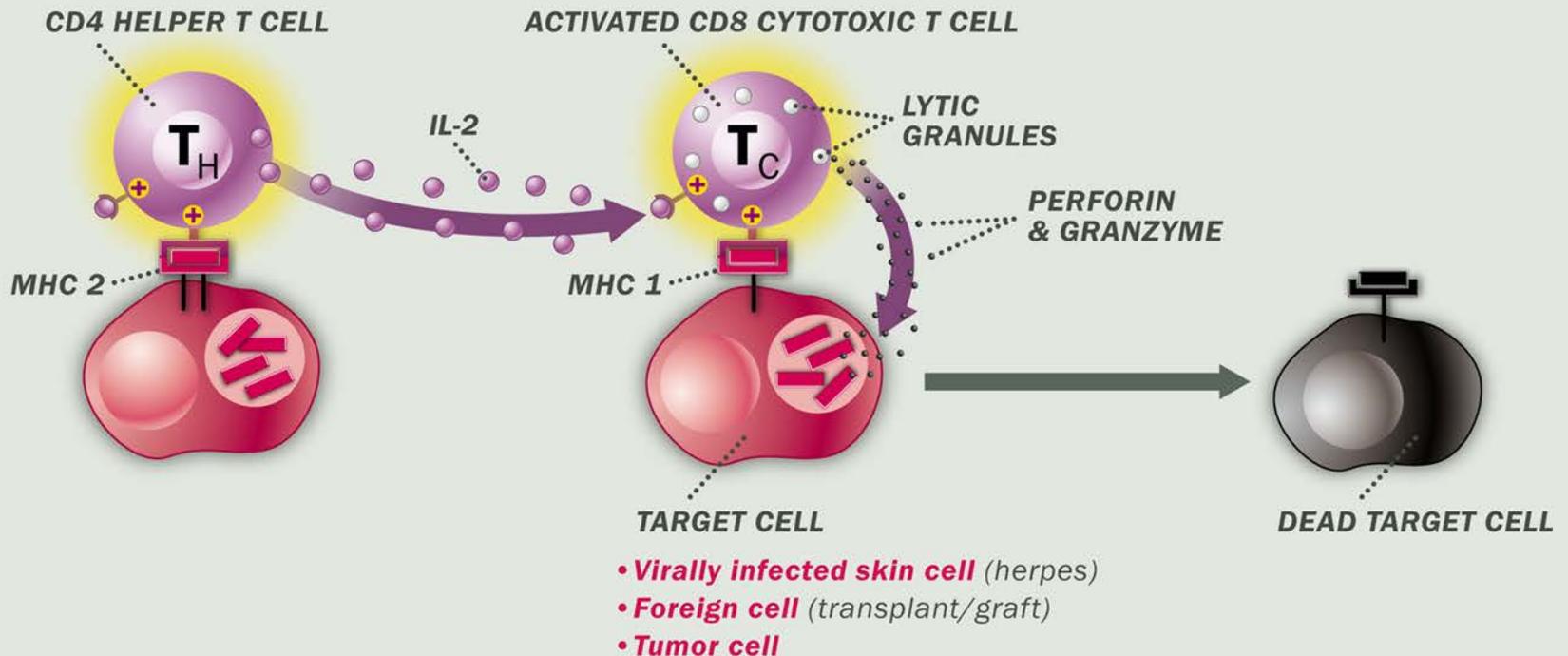
Antigen fragment presented on surface of APC in association with an MHC Class 2 molecule, activates the CD4 Helper T cell, which releases IL-2 cytokine.



Role of T cells

Activated Cytotoxic T cell Kills Target Cell

The IL-2 cytokine activates the CD8 Cytotoxic T cell which releases perforin and granzyme, killing the target cell.

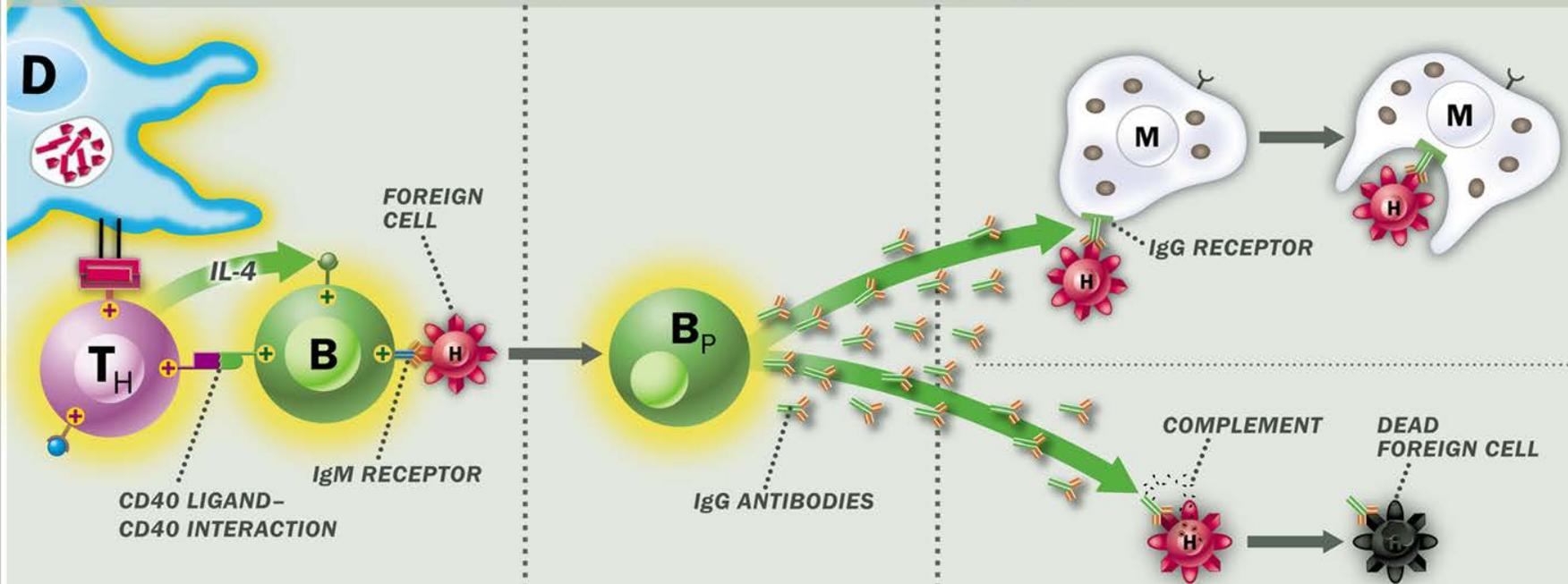


Role of B cells

Antigen binding to IgM receptor, IL-4 and CD40 ligand - CD40 interaction activates B cells

B cells mature into antibody-producing plasma cells

IgG antibodies bind to IgG receptors on macrophages, enhancing phagocytosis



Antibodies that have bound antigen can fix complement and activate it, resulting in the direct killing of foreign cells

“Cross-Talk” between the Innate and Adaptive Immune Systems

Cytokines and Their Functions

What Are Cytokines?

- ➔ Soluble protein molecules secreted by cells of the innate and adaptive immune system that affect the behavior of other cells.

- ➔ Cytokines exert their effect on the cells that they target via specific cytokine receptors expressed on the surface of their target cells.

“Cross-Talk” between the Innate and Adaptive Immune Systems

Cytokines and Their Functions

EXAMPLES OF CYTOKINES

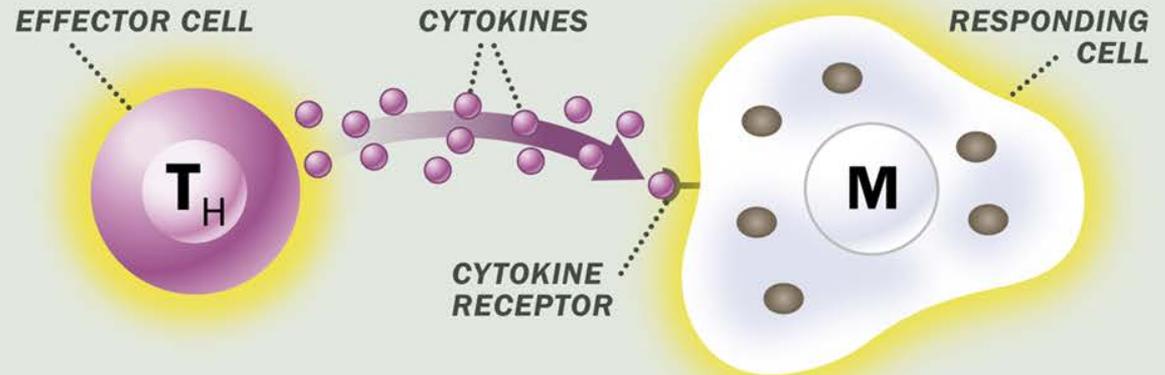
CYTOKINE	MAJOR PRODUCER CELLS	ACTION
IL-1: Interleukin 1	<ul style="list-style-type: none">• Macrophages	<ul style="list-style-type: none">• Causes fever
IL-6: Interleukin 6	<ul style="list-style-type: none">• T cells• Macrophages	<ul style="list-style-type: none">• Causes inflammation• Causes B cell activation (differentiation to plasma cells)
TNF-α: Tumor Necrosis Factor alpha	<ul style="list-style-type: none">• Macrophages• NK cells• T cells	<ul style="list-style-type: none">• Causes inflammation• Causes aches and pains
IL-12: Interleukin 12	<ul style="list-style-type: none">• Dendritic cells• Macrophages	<ul style="list-style-type: none">• Promotes IFN-γ production• Activates NK cells
IFN-γ: Interferon gamma	<ul style="list-style-type: none">• Dendritic cells• NK cells• T cells	<ul style="list-style-type: none">• Activates macrophages and NK cells
IL-2: Interleukin 2	<ul style="list-style-type: none">• T cells	<ul style="list-style-type: none">• Activates T cell proliferation

“Cross-Talk” Between Cells of the Immune System Occurs in Two Ways

1

FOR EXAMPLE $IFN-\gamma$, $TNF-\alpha$, $IL-6$

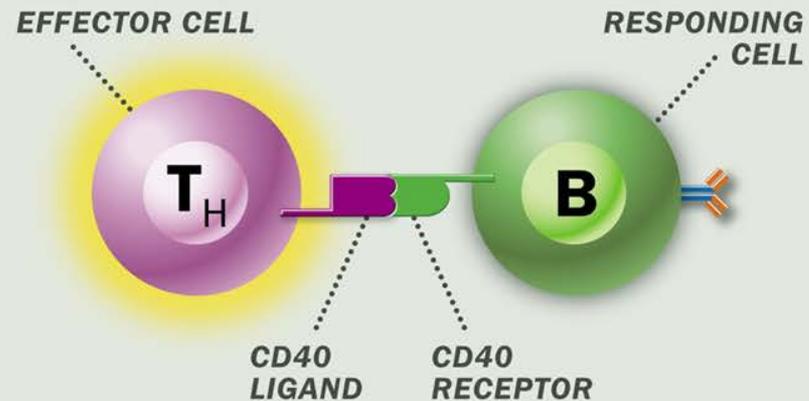
Via secreted cytokines.



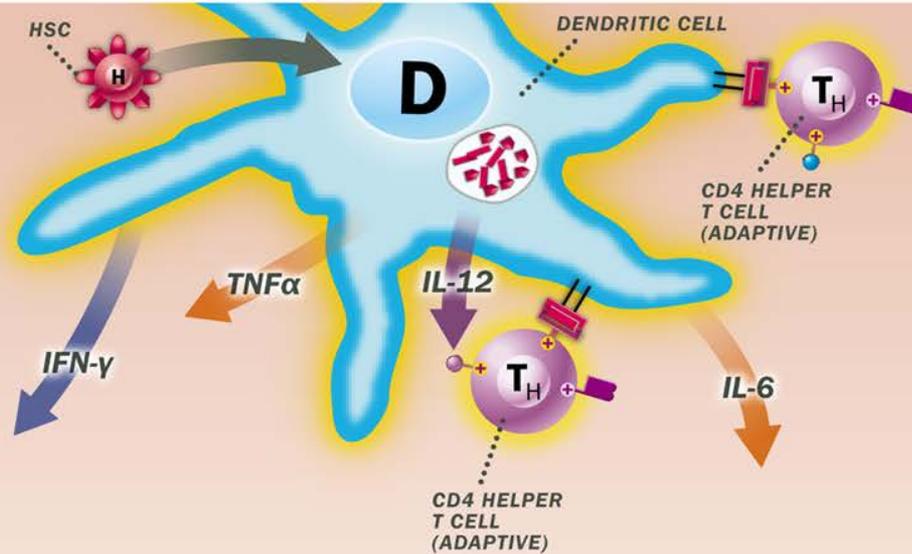
2

FOR EXAMPLE **CD40 LIGAND-CD40 INTERACTION**

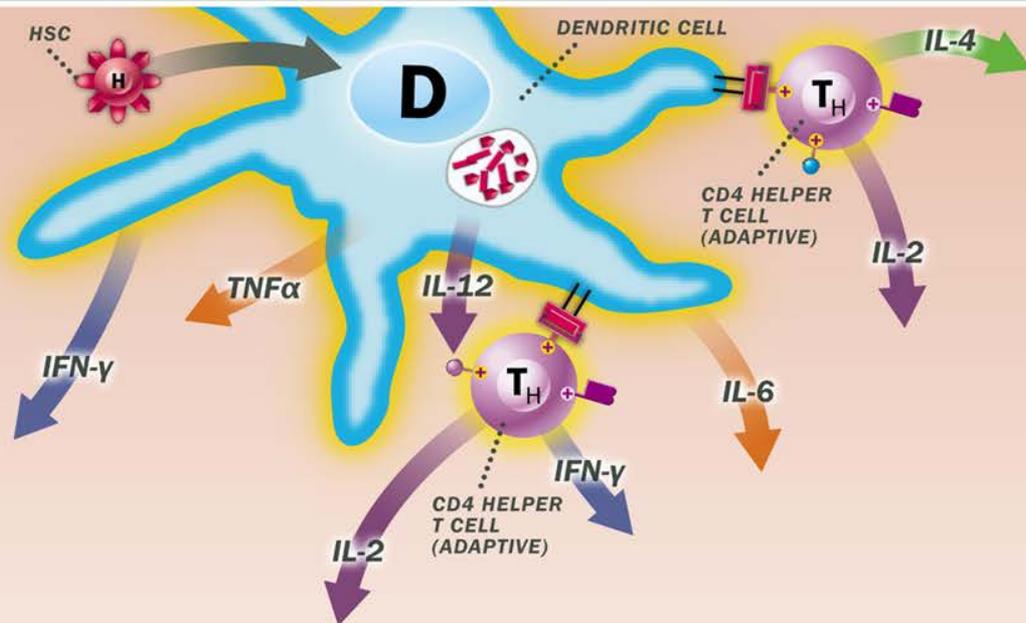
Via cell-to-cell interaction involving a ligand and receptor pair.



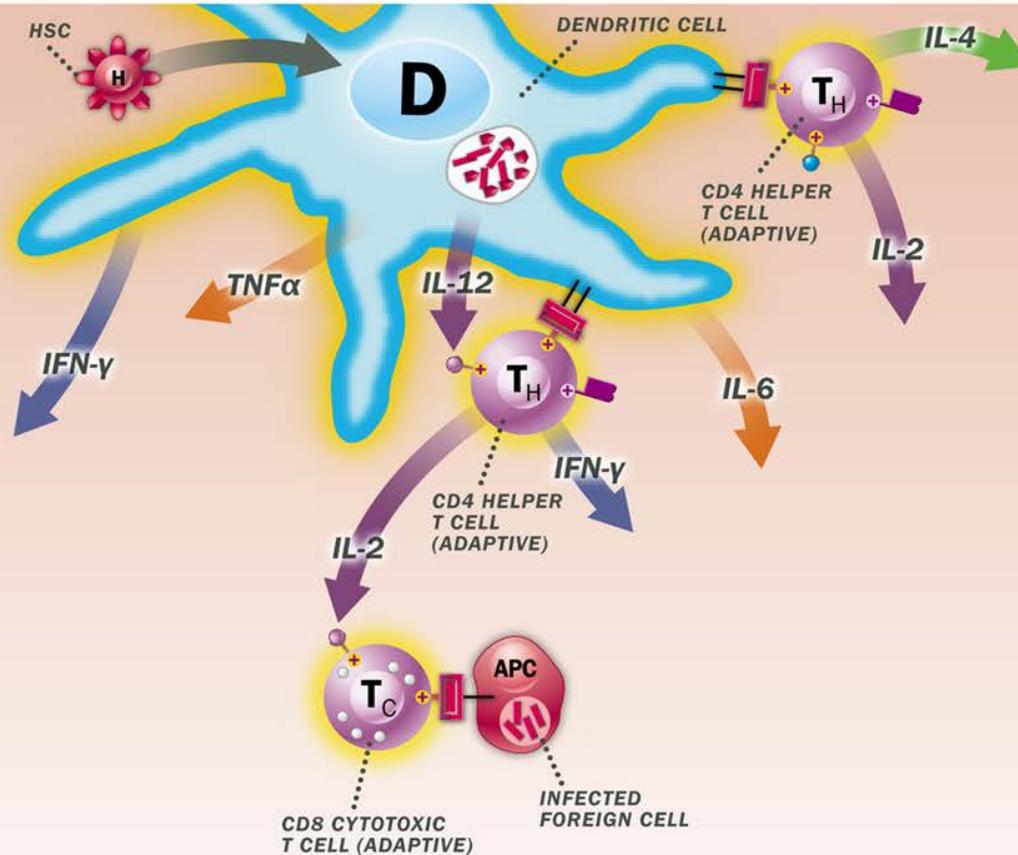
Dendritic Cells Present Antigen Fragments to CD4 Helper T cells and Secrete Cytokines IL-12, IL-6, TNF- α and IFN- γ



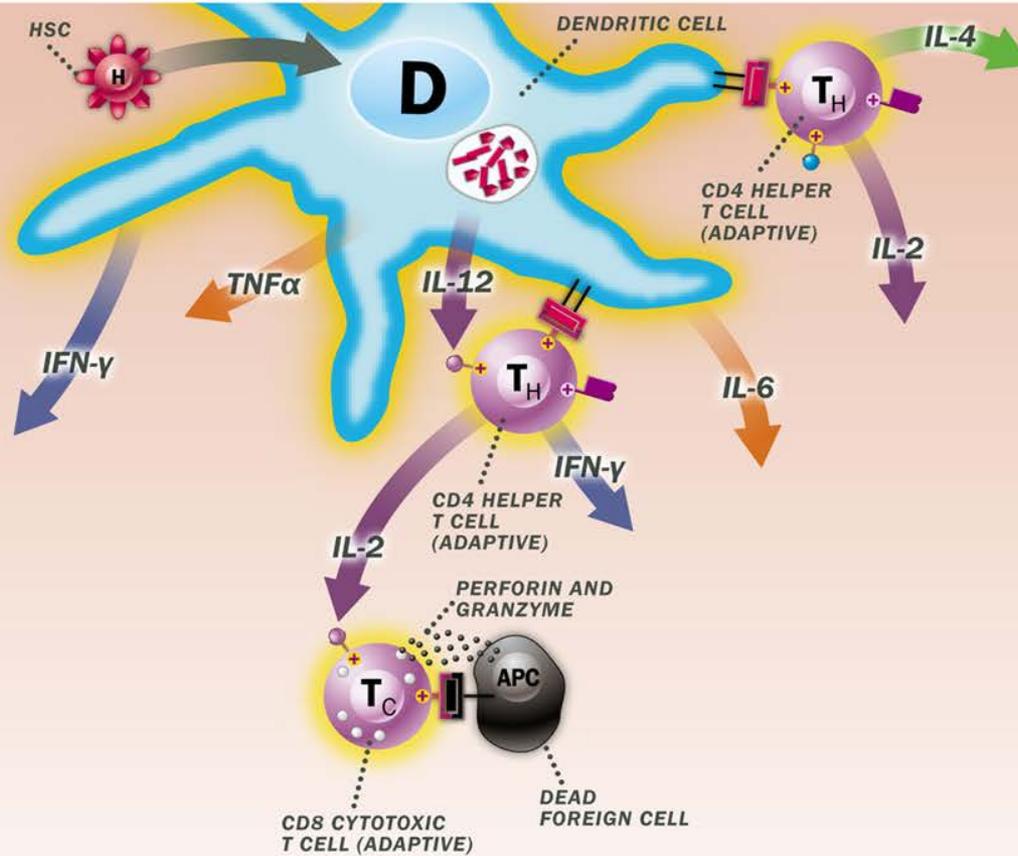
These Dendritic Cell Cytokines (i.e. IL-12) and Cell Products Act on CD4 Helper T cells, Causing Them to Secrete Cytokines IL-2, IL-4 and IFN- γ



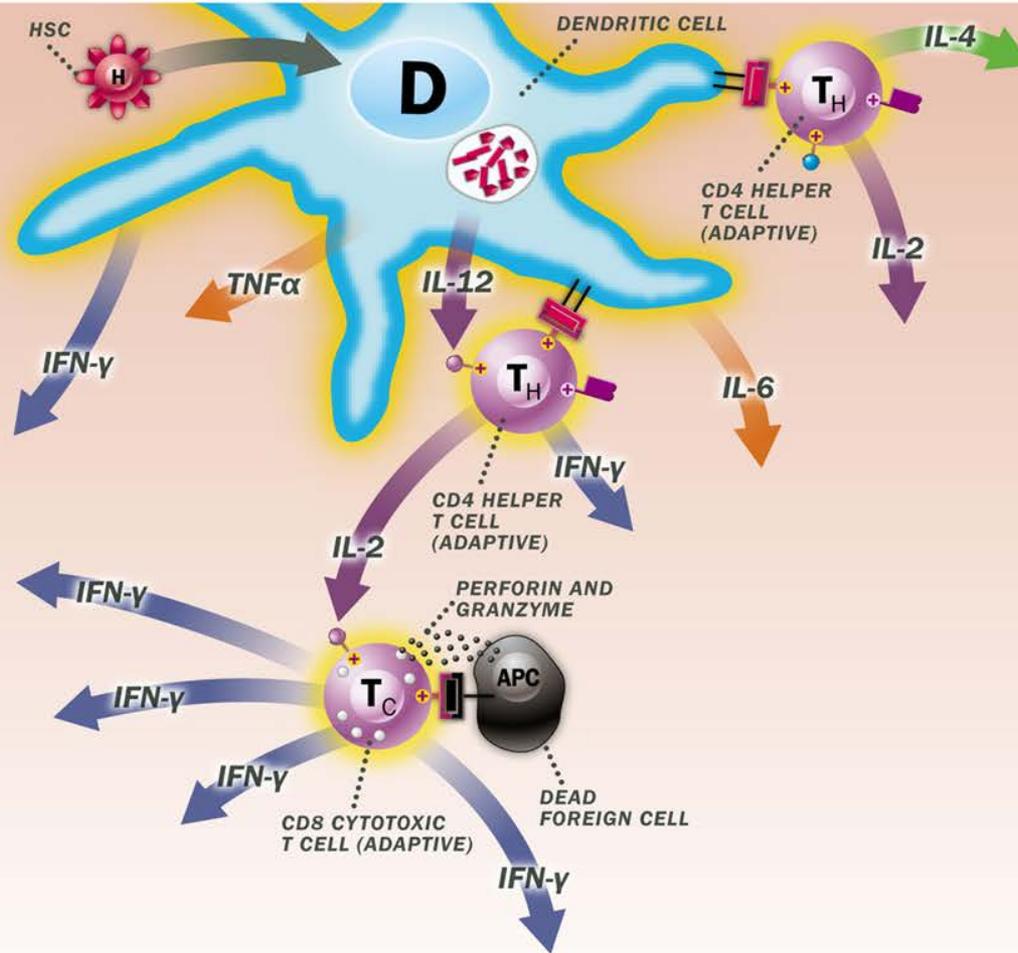
The IL-2 Cytokine Secreted by the CD4 Helper T cell Acts on the CD8 Cytotoxic T cell, which Recognizes the Target Infected (Foreign) Cell



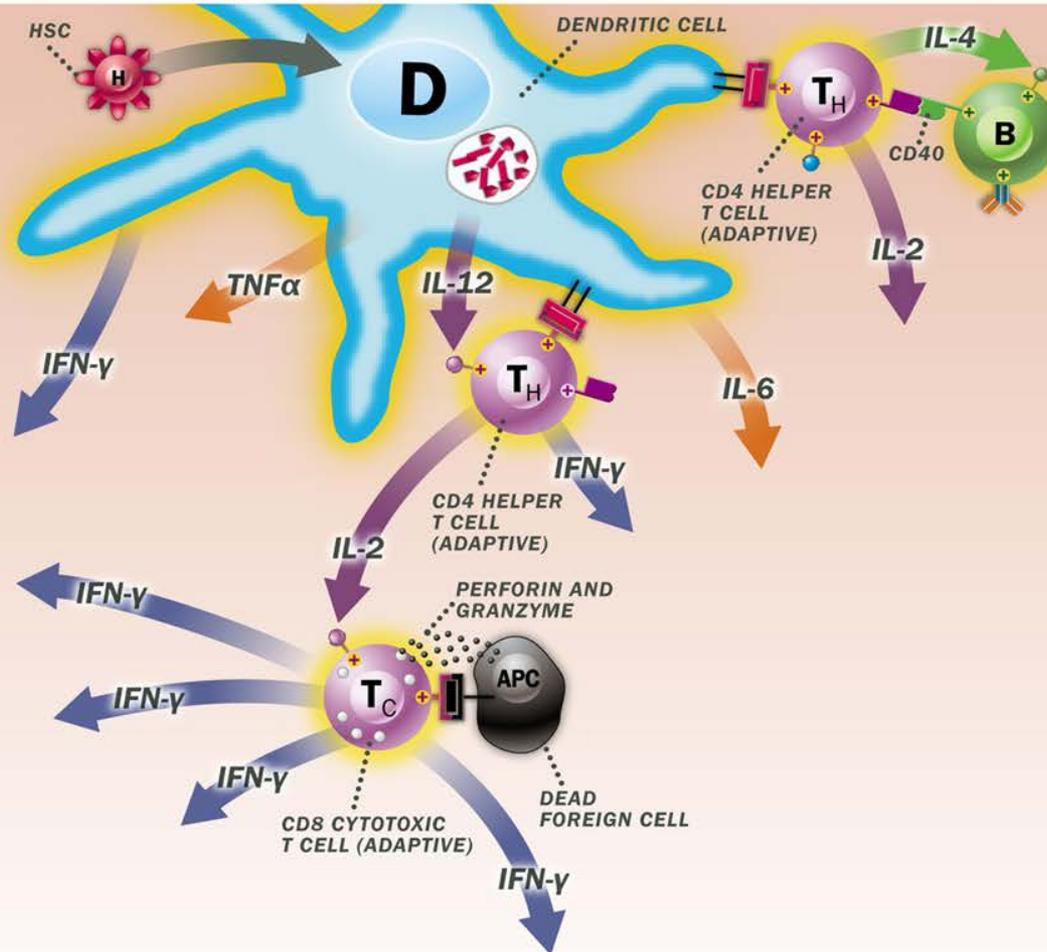
The CD8 Cytotoxic T cell Releases Perforin and Granzyme, Killing the Target Cell



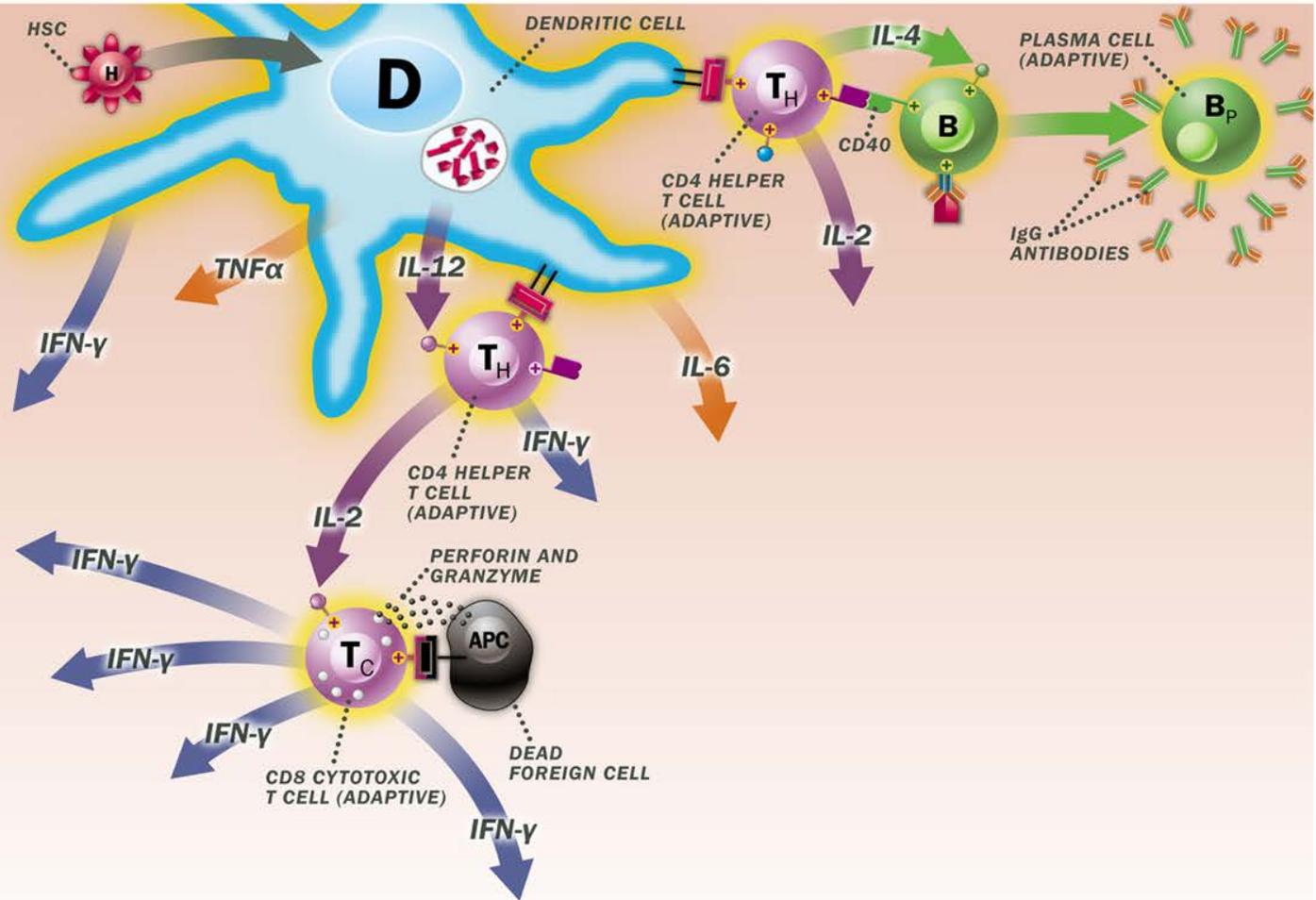
The Activated CD8 Cytotoxic T cell Also Secretes Large Amounts of Cytokine INF- γ



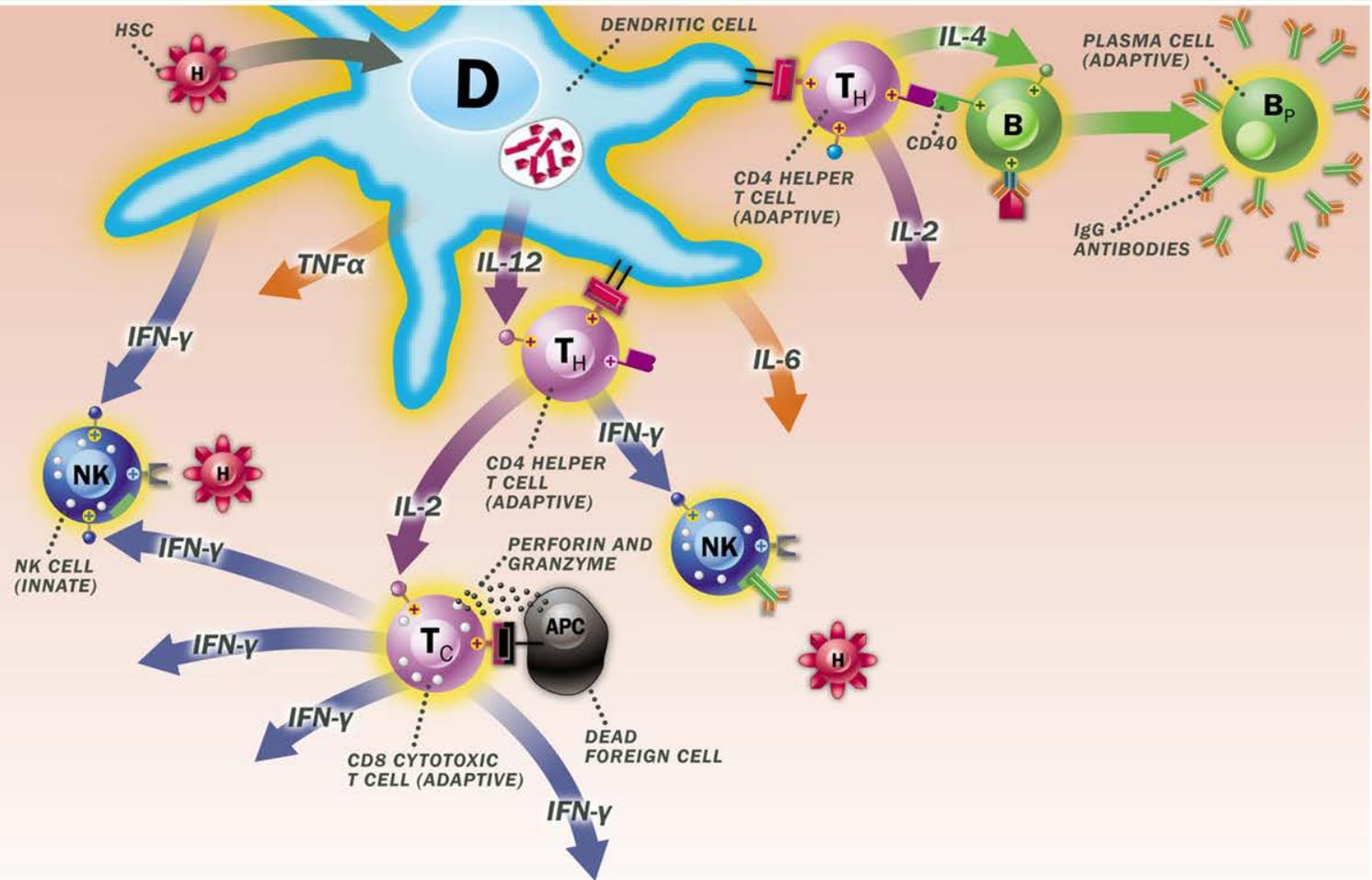
The CD4 Helper T cell Activates a Naïve B cell that Has Bound Antigen Via Interaction Between the CD40 Ligand and Its Receptor and the Release of Cytokines Such as IL-4



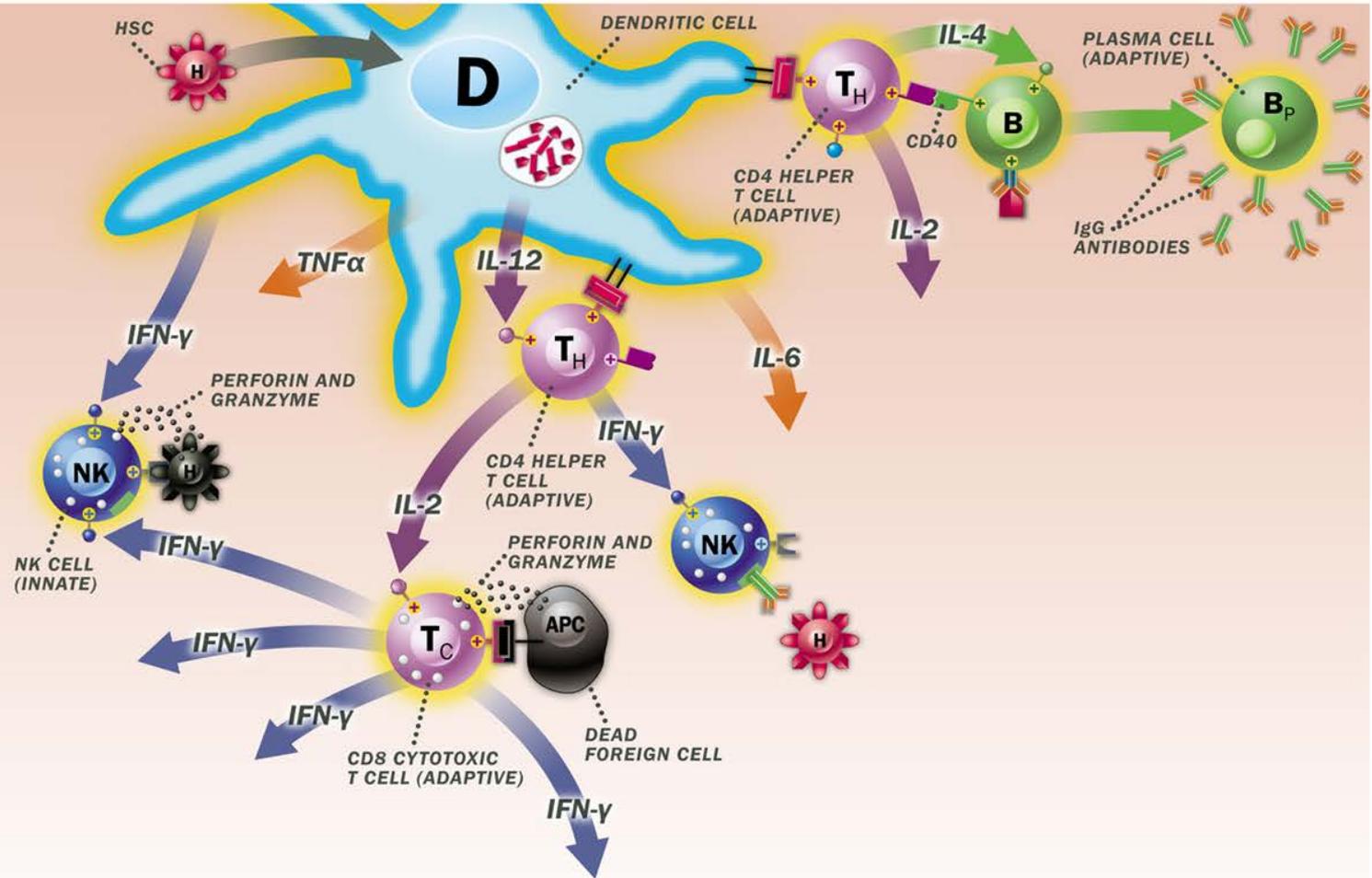
The Activated B cell Matures into an Antibody Producing Plasma Cell



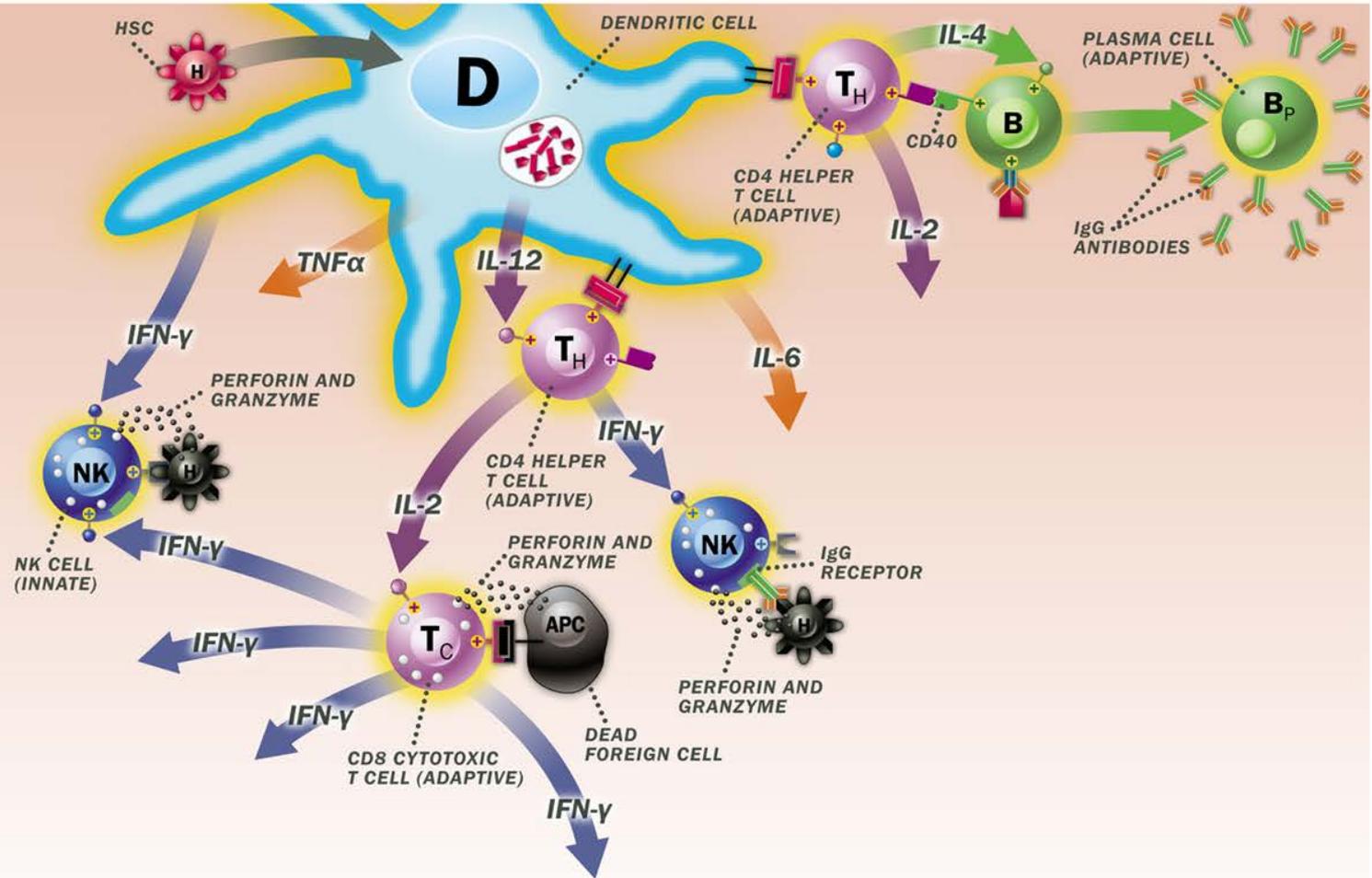
Cytokine IFN- γ Secreted by CD4 Helper and CD8 Cytotoxic T cells and Dendritic Cells Increases the Killing Activity of NK Cells



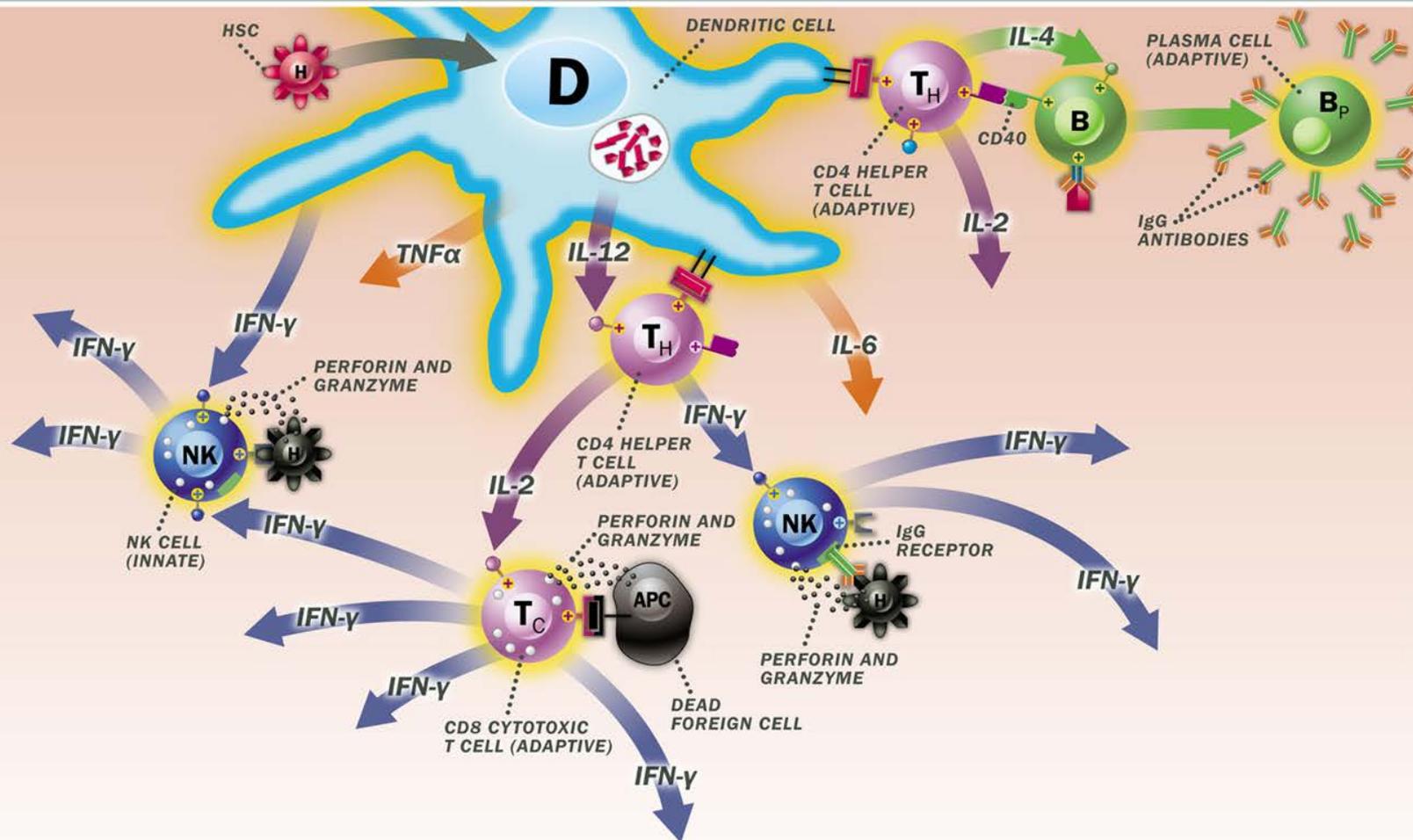
The NK Cell Receptor Recognizes Antigen on the Foreign Cell Causing the NK Cell to Release Perforin and Granzyme, Killing the Target Cell



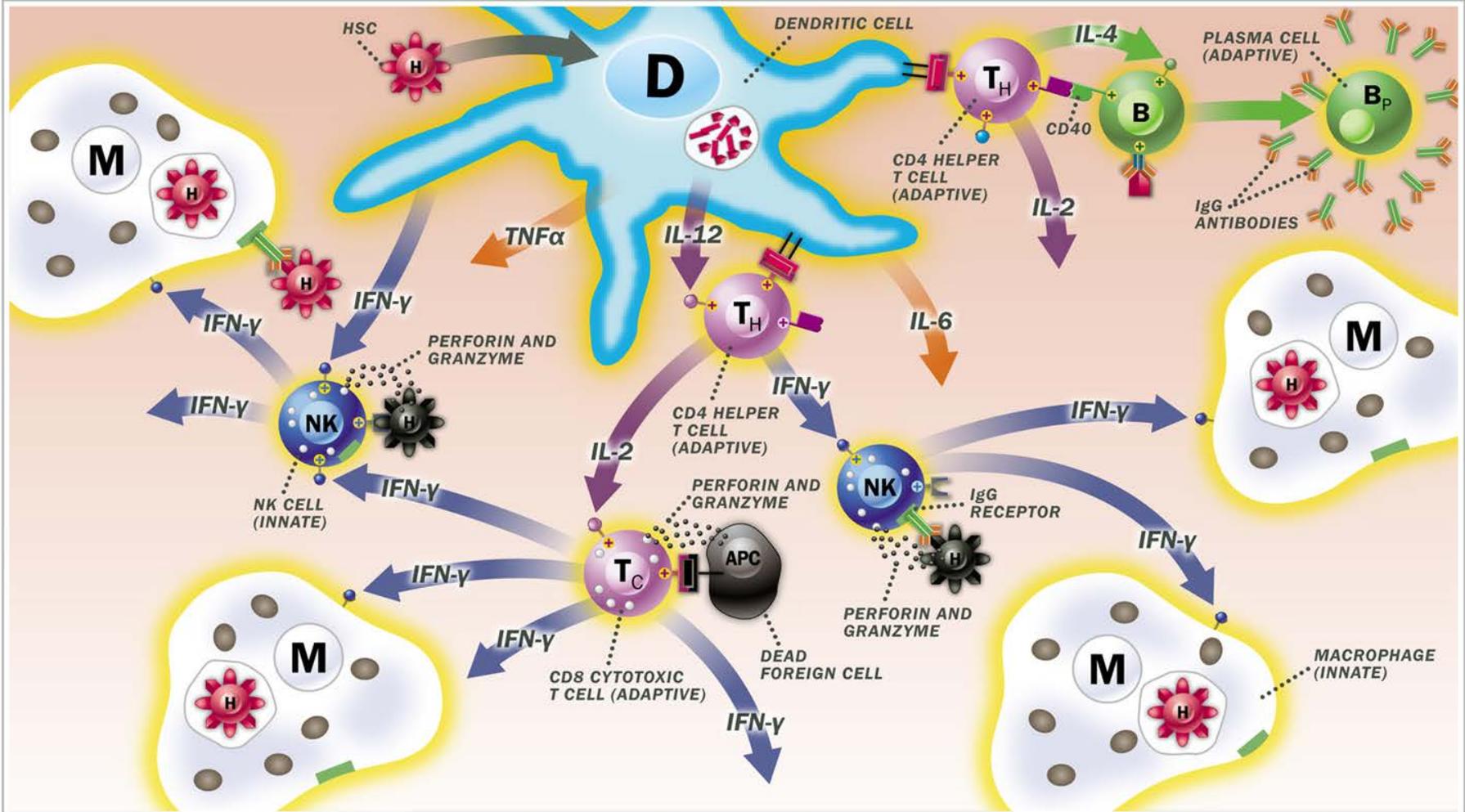
Antibodies Can Also Dock in IgG Receptors on the NK Cell, Bridging the NK Cell to the Target Cell and Enabling Killing of the Target Cell



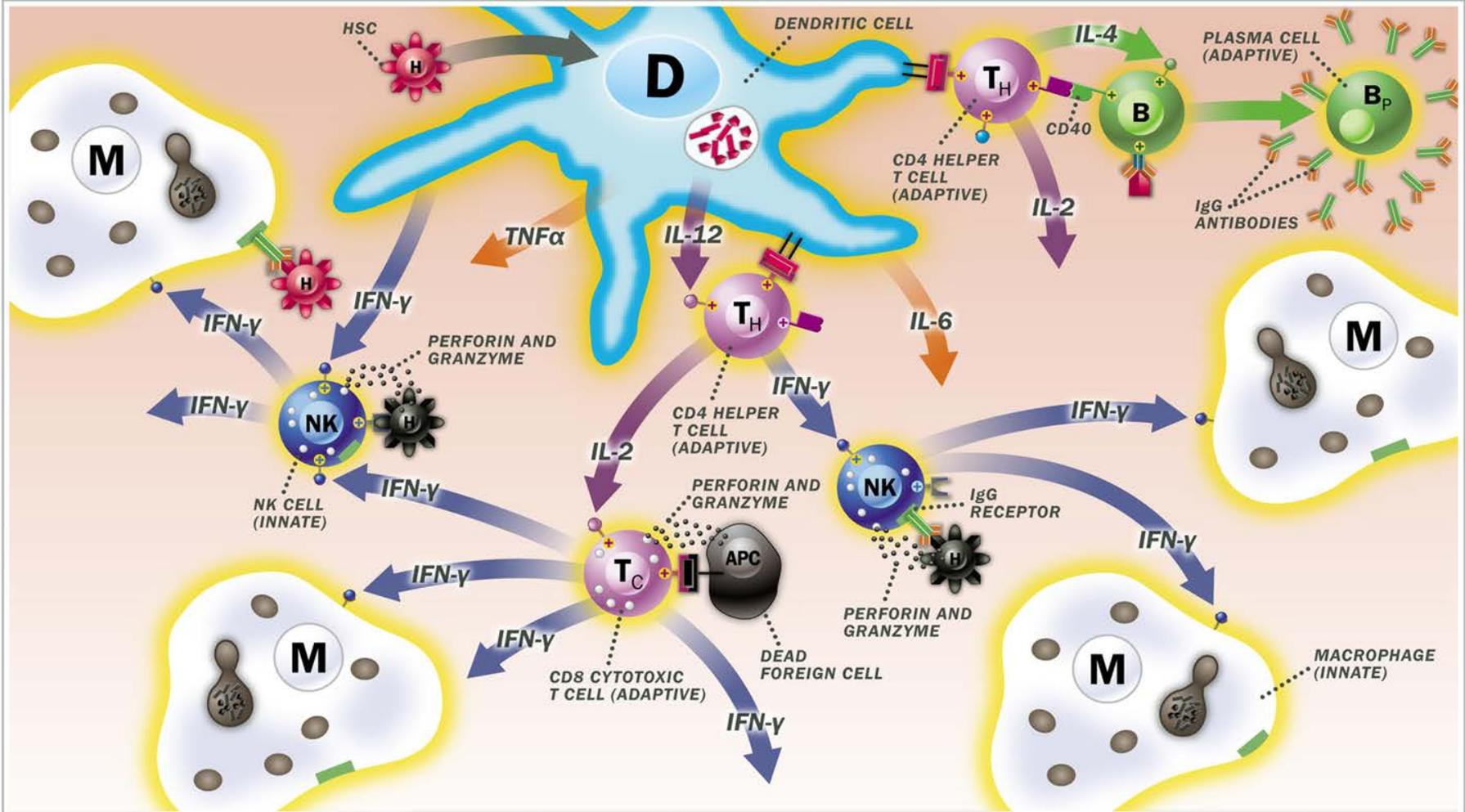
Activated NK Cells Secrete Large Amounts of Cytokine IFN- γ



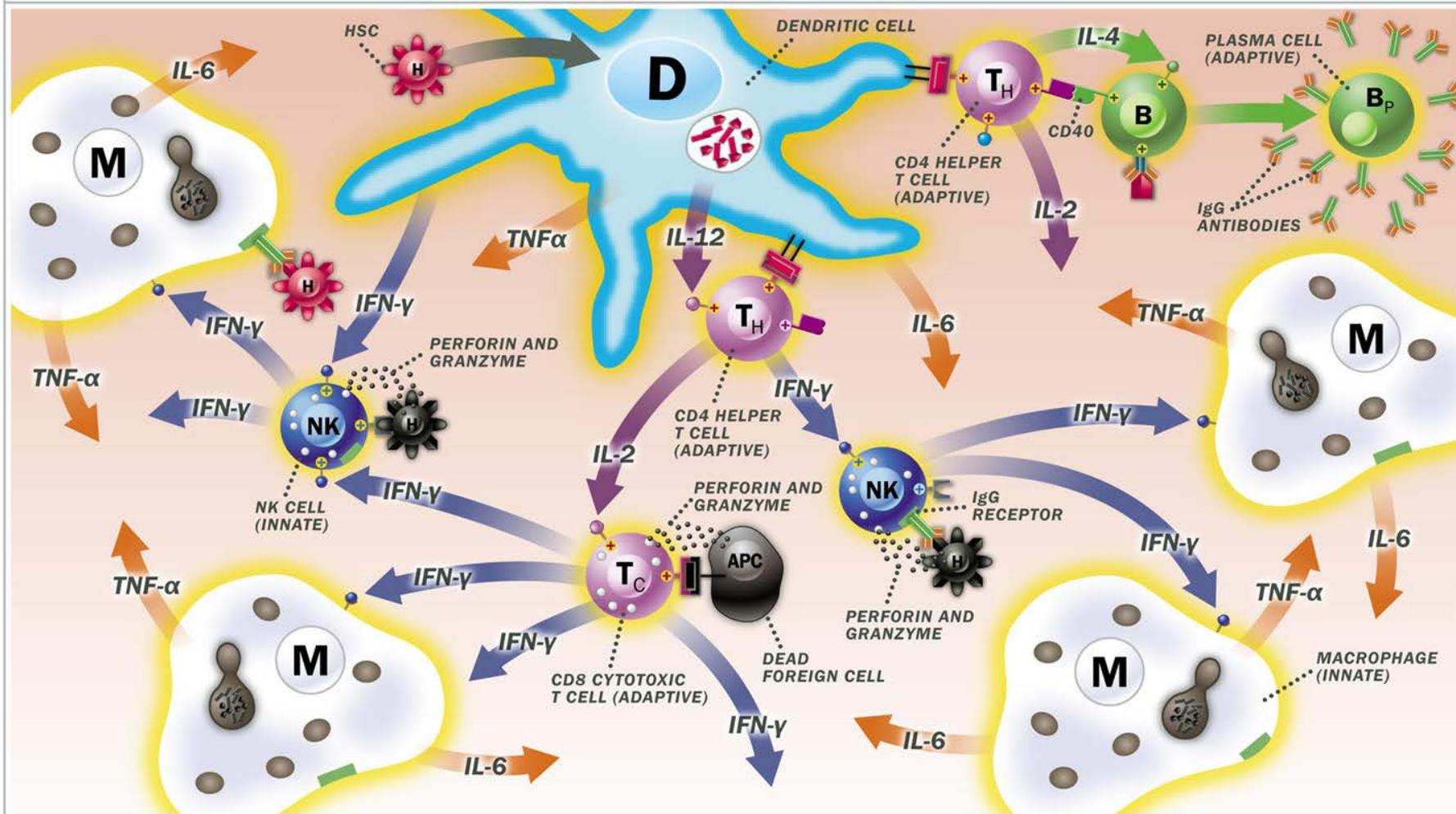
Cytokine IFN- γ Activates Macrophages, Causing Them to Become More Cytotoxic



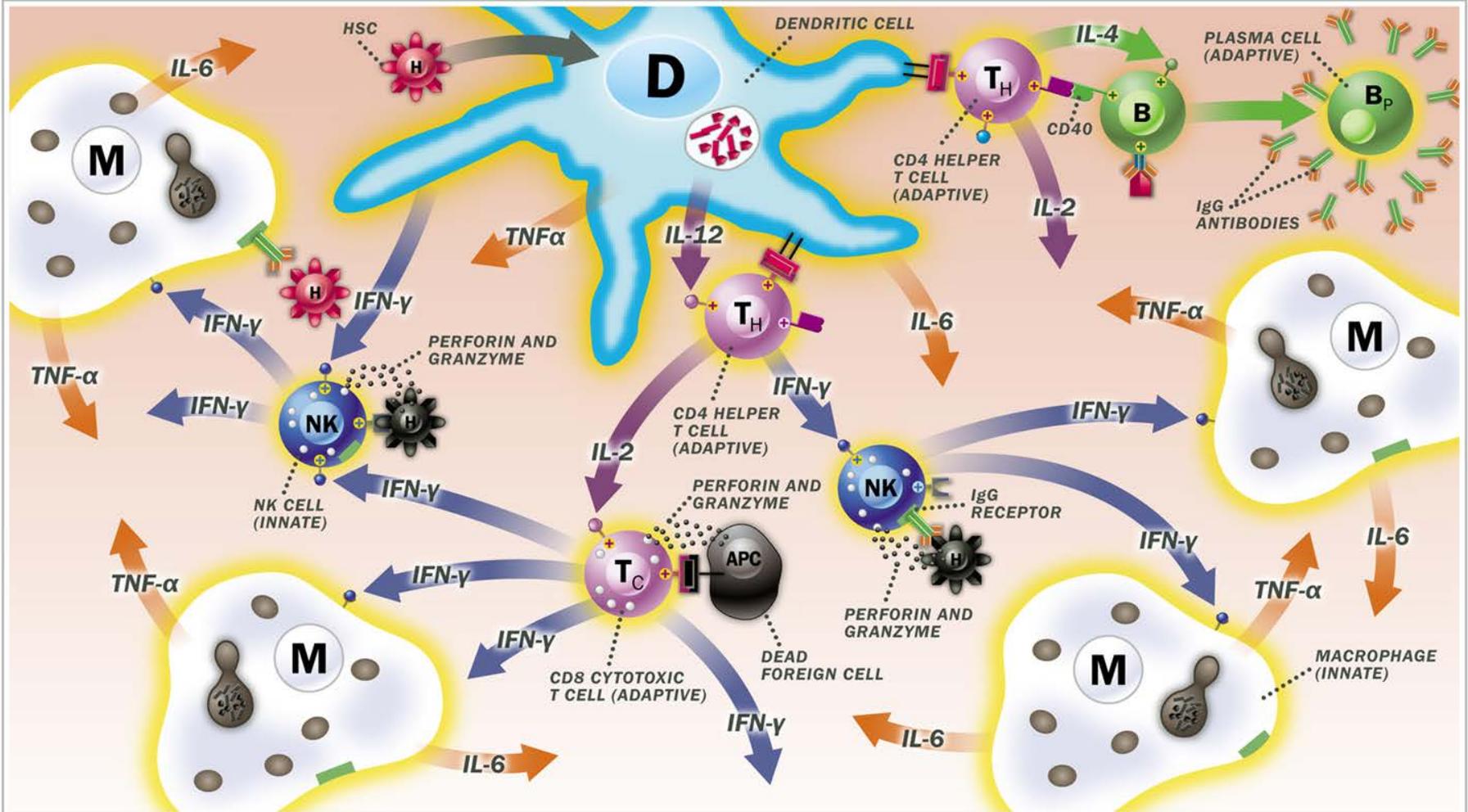
Lysosomes within the Activated Macrophages Fuse with Vesicles Containing Foreign Cells and Release Enzymes Killing the Target Cell



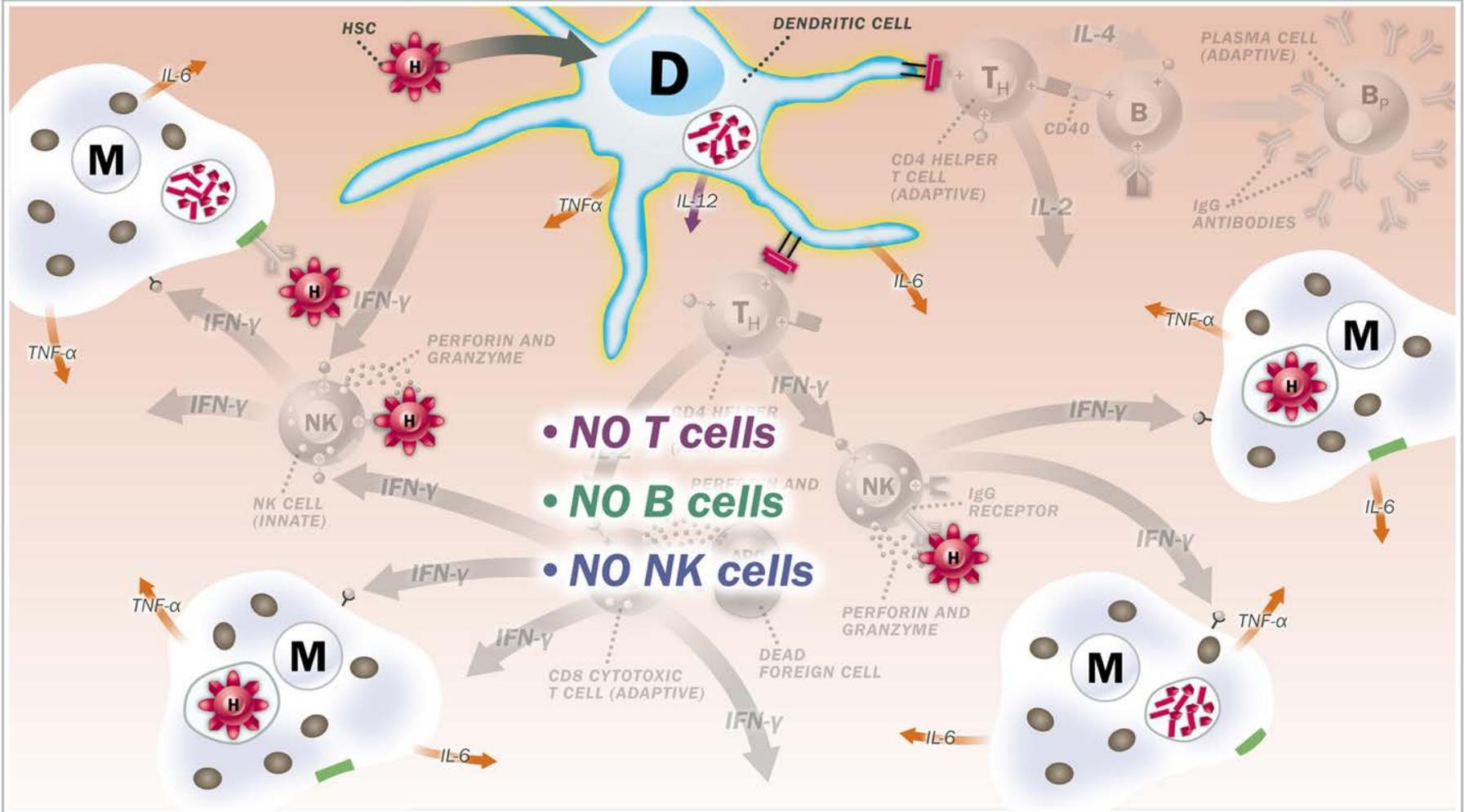
Activated Macrophages Release Cytokines TNF- α and IL-6



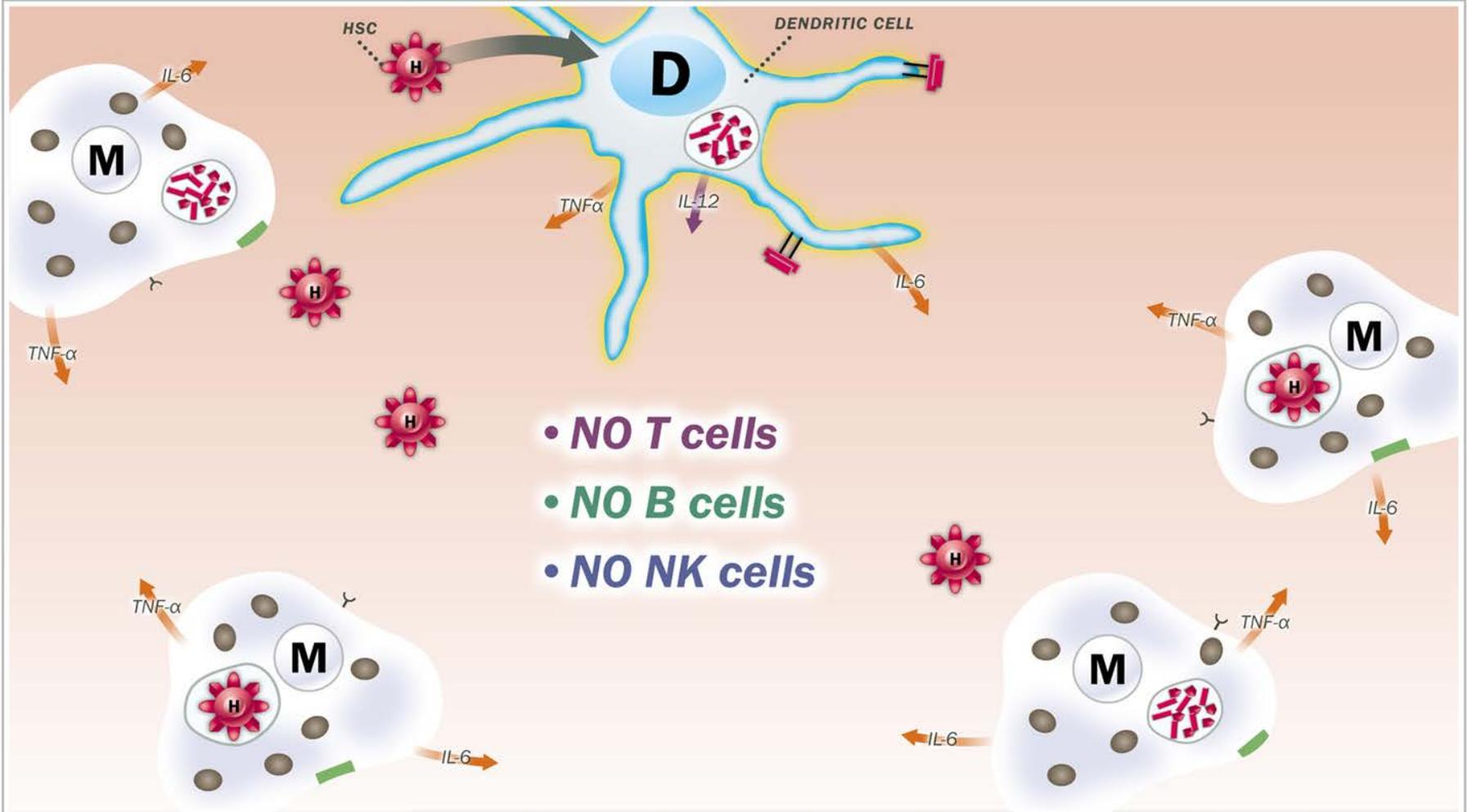
Cytokine IFN- γ Is Critical to the Role of Macrophages – It Is Often Called a Macrophage Activating Factor (MAF)



Immunodeficient NOG Mice Claimed in the '055 Patent Have No T cells, B cells or NK cells, and Exhibit Reduced Dendritic Cell and Macrophage Activity



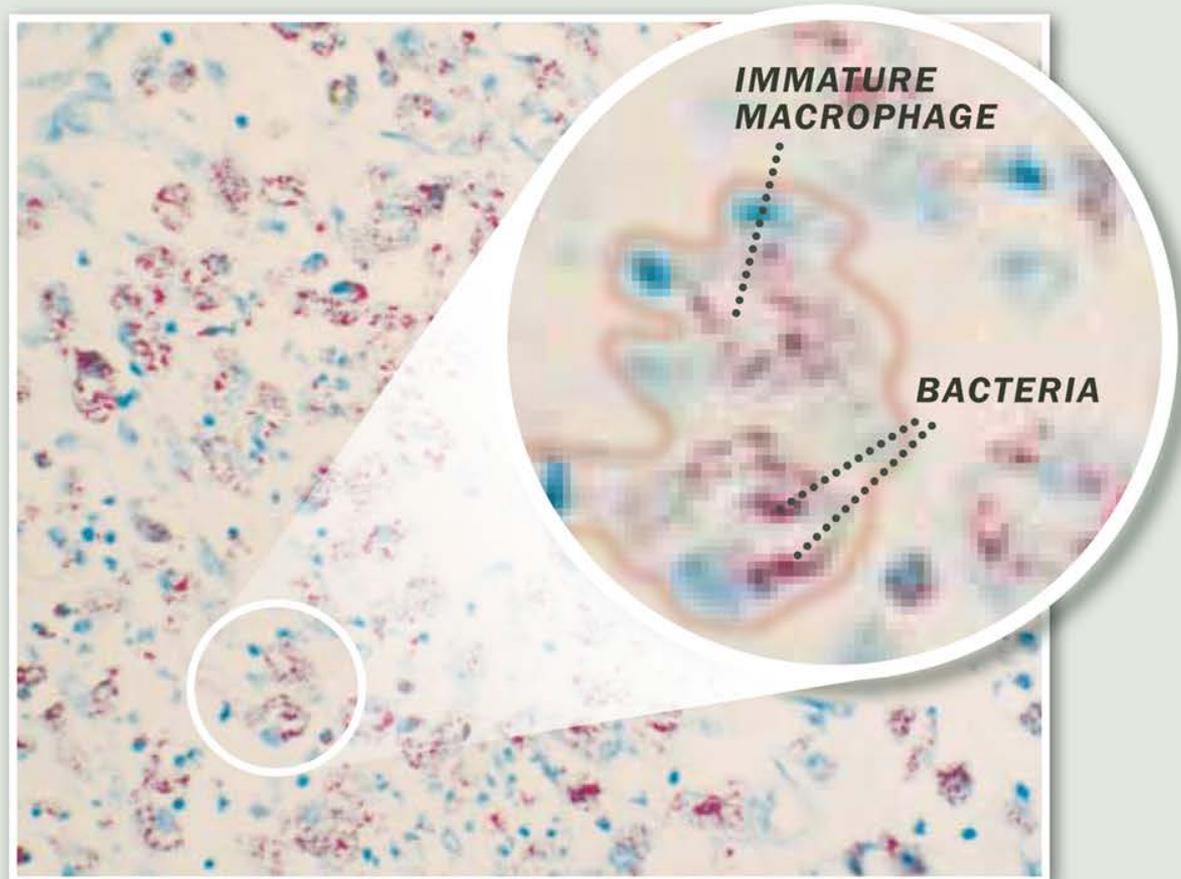
Immunodeficient NOG Mice Claimed in the '055 Patent Have No T cells, B cells or NK cells, and Exhibit Reduced Dendritic Cell and Macrophage Activity



Deficiency in IFN- γ Production and IFN- γ Receptors, Results in a Reduction of Macrophage Function

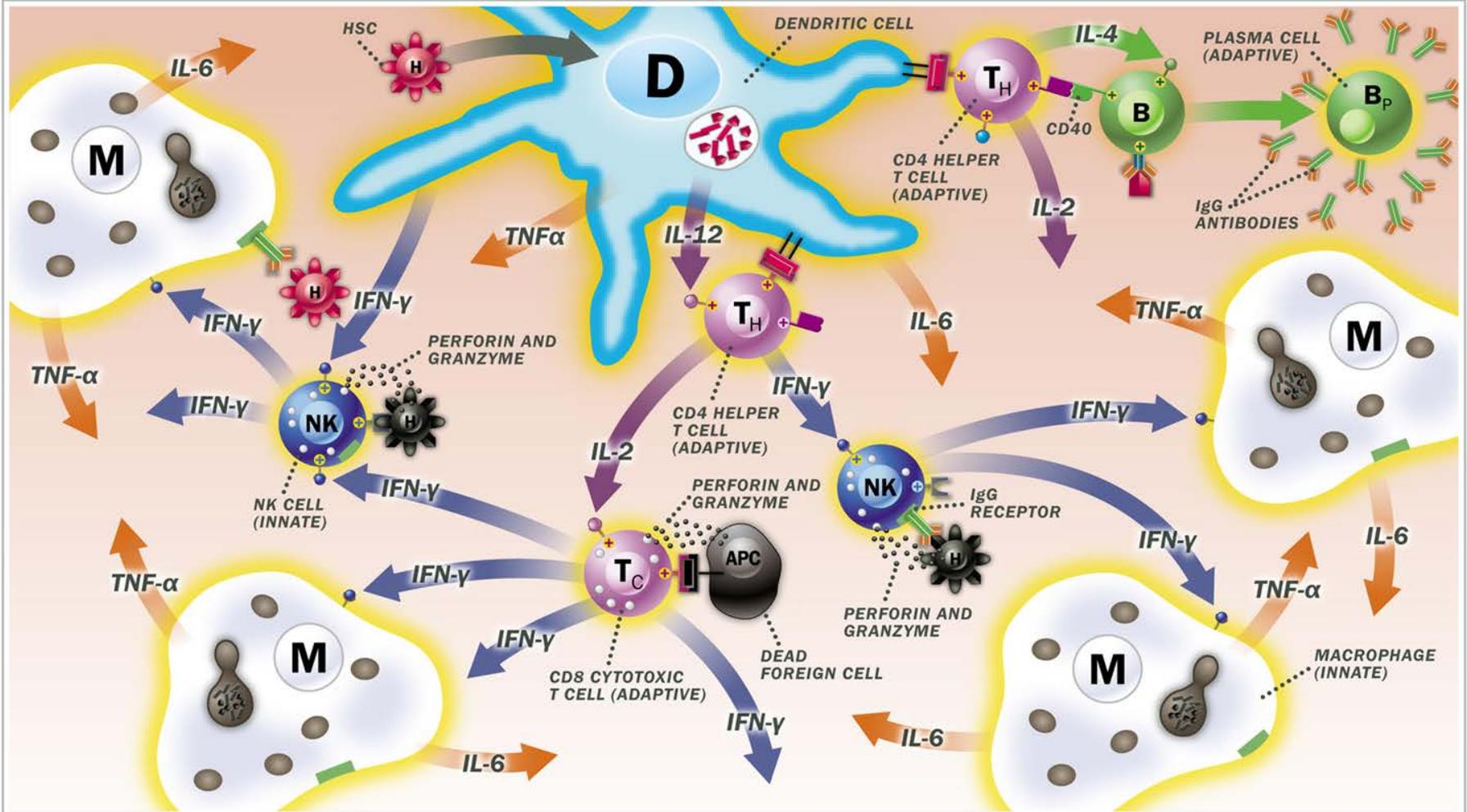
The ability of macrophages to ingest and eliminate foreign cells and particles is highly compromised

Interferon- γ -Receptor Deficiency in an Infant with Fatal Bacille Calmette-Guérin (attenuated TB vaccine) Infection



Lymph-node biopsy shows immature macrophages filled with acid-fast bacilli

Fully Operating Immune System of a Normal Mouse



A Normal Mouse Compared to a NOG Mouse

Response to Engraftment of Human Stem Cells (HSCs)

NORMAL MOUSE:

Cells of the mouse's immune system recognize the human stem cell as foreign and destroy it.

MOUSE IMMUNE CELLS

- T cells
- B cells
- NK cells
- Dendritic cells
- Macrophages

DEAD STEM CELL



A Normal Mouse Compared to a NOG Mouse

Response to Engraftment of Human Stem Cells (HSCs)

NORMAL MOUSE:

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DEAD STEM CELL



NOG MOUSE:

Absence of T, B, and NK cells coupled with reduced dendritic cell and macrophage activity does not allow the NOG mouse to eliminate the human stem cell.

MOUSE IMMUNE CELLS

- ~~T cells~~
- ~~B cells~~
- ~~NK cells~~
- ↓ Dendritic cells
- ↓ Macrophage function



A Normal Mouse Compared to a NOG Mouse

Response to Engraftment of Human Stem Cells (HSCs)

NORMAL MOUSE:		
<p>Cells of the mouse's immune system recognize the human stem cell as foreign and destroy it.</p>	<p>MOUSE IMMUNE CELLS</p> <ul style="list-style-type: none"> • T cells • B cells • NK cells • Dendritic cells • Macrophages 	<p>DEAD STEM CELL</p> 
NOG MOUSE:		
<p>Absence of T, B, and NK cells coupled with reduced dendritic cell and macrophage activity does not allow the NOG mouse to eliminate the human stem cell.</p> <p>The human stem cell proliferates and differentiates, repopulating the mouse with a human immune system.</p>	<p>MOUSE IMMUNE CELLS</p> <ul style="list-style-type: none"> • T cells • B cells • NK cells ↓ Dendritic cells ↓ Macrophage function 	<p>HUMAN IMMUNE CELLS</p> 