IInd Internal Portion

CLASS : II M.Sc ZOOLOGY PAPER - DVOLOPMENTAL BIOLOGY and IMMUNOLOGY Sub Code: 18KP3Z10

UNIT : III

Developmental Biology and Human welfare

- The main **purpose** of an economic **welfare** system is to assist citizens who are not able to support themselves or their families due to unemployment, underemployment, hardship, unskilled labor capacity, disability, or other similar reasons.
- In many cases, elderly persons and single parents may also be eligible for aid.
- Developing countries are increasingly improving their capacity to use scientific and technical knowledge to solve local problems.
- They are investing in communication infrastructure and improving technology policies. For such measures to be effective, those countries also need greater access to the world's pool of knowledge.
- It is the field of biology that studies the processes by which multicellular organisms grow and develop, controlled by their genes.
- It involves the study of mechanisms of **d**evelopment, differentiation, and growth in animals and plants at the molecular, cellular, genetic and evolutionary levels.

STRUCTURE of SPERM

Introduction

- **Sperm** is the male reproductive **cell**, or gamete, in anisogamous forms of sexual reproduction (Anisogamy is the form of sexual reproduction that involves the union or fusion of two gametes, which differ in size and /or form. the smaller gamete is considered to be mail)
- The human sperm cell only survive in warm environments; once it leaves the male body the sperm's survival likelihood is reduced and it may die, thereby decreasing the total sperm quality.
- The human **sperm cell** is haploid, so that its 23 chromosomes can join the 23 chromosomes of the female egg to form a diploid **cell**.

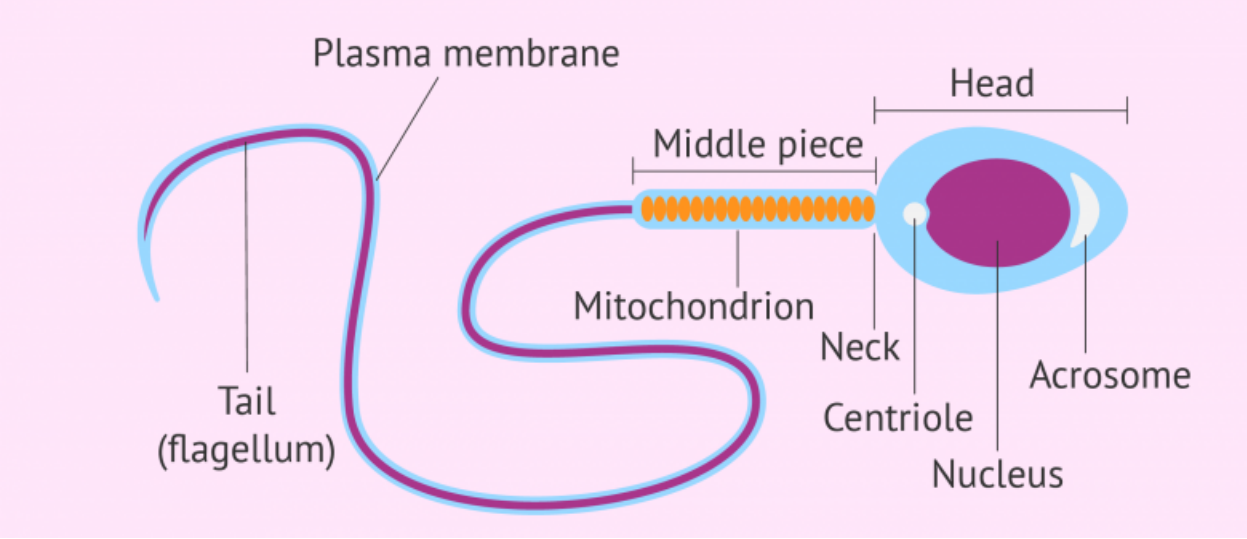
Colour of the Sperm

- Healthy semen is usually white or whitish **gray** in colour. If the semen **changes colour**, you may wonder if something is wrong with your health.

- **Yellow** semen may be nothing to worry about, but it may also be a sign of an underlying medical condition.

A sperm has three main parts:

- Sperm is composed of a head, neck, middle piece and tail. Whole body of sperm is enclosed by plasma membrane.



Head

- It has a compact nucleus with only chromatic substance and is surrounded by only a thin rim of cytoplasm.
- Above the nucleus lies a cap-like structure called the acrosome, formed by modification of the Golgi body, which secretes the enzyme **spermlysin (hyaluronidase, corona-penetrating enzyme, zona eyesin, or aerosin), that are necessary for fertilization.**
- The nucleus holds the DNA of the cell. The head contains as enlongated haploid nucleus, the anterior portion is covered by a cap-like structure called as acrosome.
- This acrosome is filled with enzymes that help fertilisation of the ovum.
- The acrosomal region experiment the acrosomal reaction, it consists in the fusion of the sperm plasma membrane with the outer acrosomal membrane.
- On the surface of the head lies a decapacitating substance which is removed before fertilisation.

Neck

- It is the smallest part and has a proximal centriole parallel to the base of the nucleus and distal centriole perpendicular to the previous one.
- The proximal centriole is present also in the mature spermatozoon; the distal centriole disappears after axoneme assembly.
- The proximal centriole enters into the egg during fertilisation and starts the first cleavage division of the egg, which has no centriole. The distal centriole gives rise to the axial filament which forms the tail and has a arrangement. A transitory membrane called the Manchette lies in the middle piece.

Sperm centrioles are important for 2 functions:

(1) To form the **sperm** flagellum and **sperm** movement and

(2) For the development of the embryo after fertilization. The **sperm** supplies the **centriole** that creates the centrosome and microtubule system of the zygote.

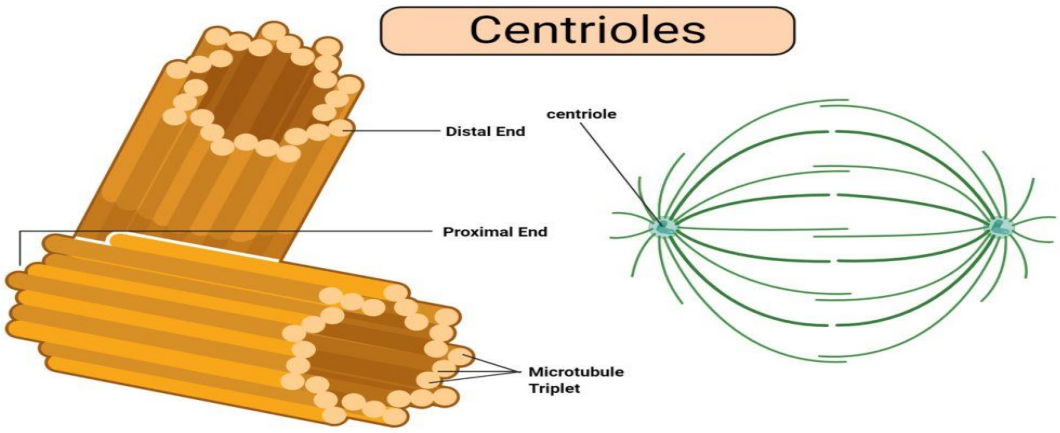


Figure: Centrioles, Image Copyright © Sagar Aryal, www.microbenotes.com

Middle piece

- The midpiece of the **sperm** is packed with mitochondria. t has 10–14 spirals of mitochondria surrounding the axial filament in the cytoplasm.
- The middle piece possesses numerous mitochondria, which produce energy for the movement of tail that facilitate sperm motility essential for fertilisation. and hence is called the powerhouse of the sperm.
- The human male ejaculates about 200 to 300 million sperms during a coitus of which, for normal fertility , at least 60% sperms must have normal shape and size and at least 40% of them must show vigorous motility.

Tail

- It is the longest part having an axial filament surrounded by cytoplasm and plasma membrane, but at the posterior end the axial filament is naked.
- It is push mechanism. The tail of the **sperm** moves like a propeller, around and around.

Sperm Motility

- Semen has an alkaline nature and the spermatozoa do not reach full motility (hypermotility) until they reach the vagina, where the alkaline pH is neutralized by acidic vaginal fluids.
- This gradual process takes 20–30 minutes. During this period, fibrinogen from the seminal vesicles forms a clot, securing and protecting the sperm.
- Just as they become hyper motile, fibrinolysin from the prostate gland dissolves the clot, allowing the sperm to progress optimally.

Spermatozoon

- The spermatozoon is characterized by a minimum of cytoplasm and the most densely packed DNA known in eukaryotes. Compared to mitotic chromosomes in somatic cells, sperm DNA is at least sixfold more highly condensed

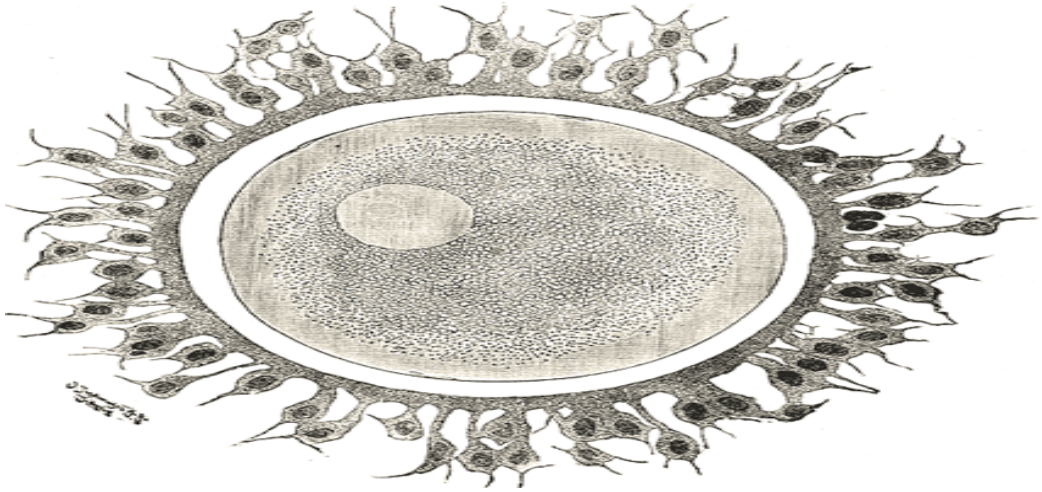
Function.

- The **main sperm function** is to reach the ovum and fuse with it to deliver **two** sub-cellular structures: (i) the male pronucleus that contains the genetic material and (ii) the centrioles that are structures that help organize the microtubule cytoskeleton.

Structure of Human Ovum

- The ova are developed from the primitive germ cells which are imbedded in the substance of the ovaries.
- Each primitive germ cell gives rise, by repeated divisions, to a number of smaller cells termed oögonia, from which the ova or primary oöcytes are developed.

Structure of the Ovum



Human ova are extremely minute, measuring about 0.2 mm. in diameter, and are enclosed within the egg follicles of the ovaries; as a rule each follicle contains a single ovum, but sometimes two or more are present. (*3 By the enlargement and subsequent rupture of a follicle at the surface of the ovary, an ovum is liberated and conveyed by the uterine tube to the cavity of the uterus. Unless it be fertilized it undergoes no further development and is discharged from the uterus, but if fertilization take place it is retained within the uterus and is developed into a new being.

In appearance and structure the ovum differs little from an ordinary cell, but distinctive names have been applied to its several parts; thus, the cell substance is known as the yolk or oöplasm, the nucleus as the germinal vesicle, and the nucleolus as the germinal spot. The ovum is enclosed within a thick, transparent envelope, the zona striata or zona pellucida, adhering to the outer surfaces of which are several layers of cells, derived from those of the follicle and collectively constituting the corona radiata.

Yolk

The yolk comprises (1) the cytoplasm of the ordinary animal cell with its spongioplasm and hyaloplasm; this is frequently termed the formative yolk; (2) the nutritive yolk or deutoplasm, which consists of numerous rounded granules of fatty and albuminoid substances imbedded in the cytoplasm. In the mammalian ovum the nutritive yolk is

extremely small in amount, and is of service in nourishing the embryo in the early stages of its development only, whereas in the egg of the bird there is sufficient to supply the chick with nutriment throughout the whole period of incubation. The nutritive yolk not only varies in amount, but in its mode of distribution within the egg; thus, in some animals it is almost uniformly distributed throughout the cytoplasm; in some it is centrally placed and is surrounded by the cytoplasm; in others it is accumulated at the lower pole of the ovum, while the cytoplasm occupies the upper pole. A centrosome and centriole are present and lie in the immediate neighborhood of the nucleus.

Germinal Vesicle.

The germinal vesicle or nucleus is a large spherical body which at first occupies a nearly central position, but becomes eccentric as the growth of the ovum proceeds. Its structure is that of an ordinary cell-nucleus, viz., it consists of a reticulum or karyomitome, the meshes of which are filled with karyoplasm, while connected with, or imbedded in, the reticulum are a number of chromatin masses or chromosomes, which may present the appearance of a skein or may assume the form of rods or loops. The nucleus is enclosed by a delicate nuclear membrane, and contains in its interior a well-defined nucleolus or germinal spot.

Coverings of the Ovum

The zona striata or zona pellucida is a thick membrane, which, under the higher powers of the microscope, is seen to be radially striated. It persists for some time after fertilization has occurred, and may serve for protection during the earlier stages of segmentation. It is not yet determined whether the zona striata is a product of the cytoplasm of the ovum or of the cells of the corona radiata, or both.

The corona radiata consists or two or three strata of cells; they are derived from the cells of the follicle, and adhere to the outer surface of the zona striata when the ovum is set free from the follicle; the cells are radially arranged around the zona, those of the innermost layer being columnar in shape. The cells of the corona radiata soon disappear; in some animals they secrete, or are replaced by, a layer of adhesive protein, which may assist in protecting and nourishing the ovum.

The phenomena attending the discharge of the ova from the follicles belong more to the ordinary functions of the ovary than to the general subject of embryology, and are therefore described with the anatomy of the ovaries.

Maturation of the Ovum.

Before an ovum can be fertilized it must undergo a process of maturation or ripening. This takes place previous to or immediately after its escape from the follicle, and consists essentially of an unequal subdivision of the ovum first into two and then into four cells. Three of the four cells are small, incapable of further development, and are termed polar bodies or polocytes, while the fourth is large, and constitutes the mature ovum. The process of maturation has not been observed in the human ovum, but has been carefully studied in the ova of some of the lower animals, to which the following description applies.

It was pointed out on page 37 that the number of chromosomes found in the nucleus is constant for all the cells in an animal of any given species, and that in man the number is probably twenty-four. This applies not only to the somatic cells but to the primitive ova and their descendants. For the purpose of illustrating the process of maturation a species may be taken in which the number of nuclear chromosomes is four. If an ovum from such be observed at the beginning of the maturation process it will be seen that the number of its chromosomes is apparently reduced to two. In reality, however, the number is doubled, since each chromosome consists of four granules grouped to form a tetrad. During the metaphase each tetrad divides into two dyads, which are equally distributed between the nuclei of the two cells formed by the first division of the ovum. One of the cells is almost as large as the original ovum, and is named the secondary oöcyte; the other is small, and is termed the first polar body.

The secondary oöcyte now undergoes subdivision, during which each dyad divides and contributes a single chromosome to the nucleus of each of the two resulting cells.

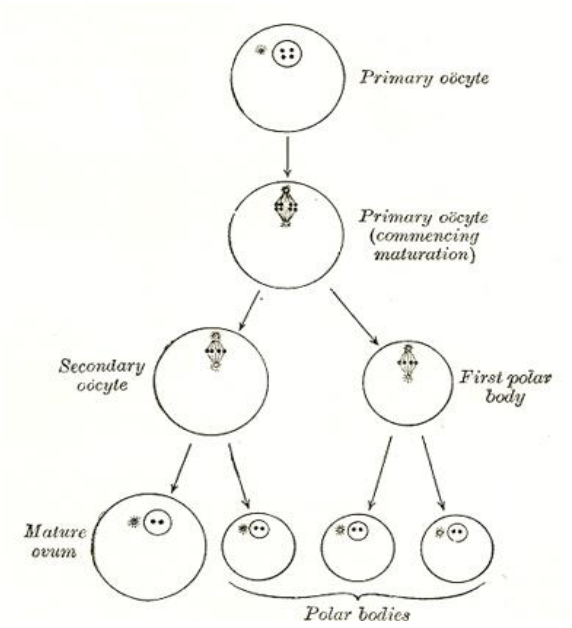


Diagram showing the reduction in number of the chromosomes in the process of aturation of the ovum.

This second division is also unequal, producing a large cell which constitutes the mature ovum, and a small cell, the second polar body. The first polar body frequently divides while the second is being formed, and as a final result four cells are produced, viz., the mature ovum and three polar bodies, each of which contains two chromosomes, i.e., one-half the number present in the nuclei of the somatic cells of members of the same species. The nucleus of the mature ovum is termed the female pronucleus.

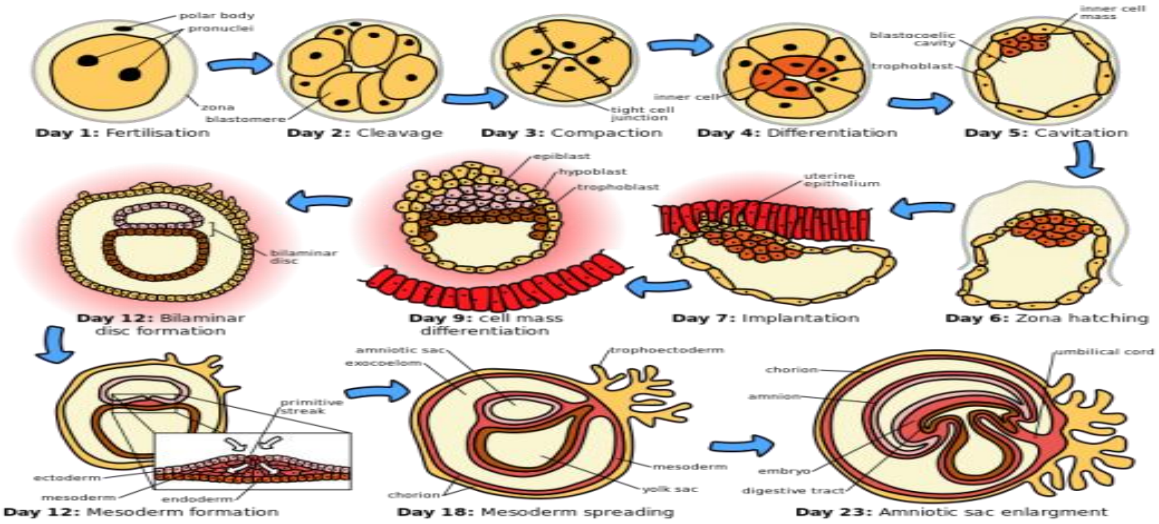
Human Embryonic Development

Human embryonic development, or human embryogenesis, refers to the development and formation of the human embryo. It is characterized by the processes of cell division and cellular differentiation of the embryo that occurs during the early stages of development. In biological terms, the development of the human body entails growth from a one-celled zygote to an adult human being. Fertilization occurs when the sperm cell successfully enters and fuses with an egg cell (ovum). The genetic material of the sperm and egg then combine to form a single cell called a zygote and the germinal stage of development commences. Embryonic development in the human covers the first eight weeks of development; at the beginning of the ninth week the embryo is termed

a fetus. Human embryology is the study of this development during the first eight weeks after fertilization.

The normal period of gestation (pregnancy) is about nine months or 40 weeks. The germinal stage refers to the time from fertilization through the development of the early embryo until implantation is completed in the uterus. The germinal stage takes around 10 days. During this stage, the zygote begins to divide, in a process called cleavage. A blastocyst is then formed and implanted in the uterus. Embryogenesis continues with the next stage of gastrulating, when the three germ layers of the embryo form in a process called histogenesis, and the processes of neurulation and organogenesis follow.

In comparison to the embryo, the fetus has more recognizable external features and a more complete set of developing organs. The entire process of embryogenesis involves coordinated spatial and temporal changes in gene expression, cell growth and cellular differentiation. A nearly identical process occurs in other species, especially among chordates.



Germinal Stage

Fertilization

Fertilization takes place when the spermatozoon has successfully entered the ovum and the two sets of genetic material carried by the gametes fuse together, resulting in the zygote

(a single diploid cell). This usually takes place in the ampulla of one of the fallopian tubes. The zygote contains the combined genetic material carried by both the male and female gametes which consists of the 23 chromosomes from the nucleus of the ovum and the 23 chromosomes from the nucleus of the sperm. The 46 chromosomes undergo changes prior to the mitotic division which leads to the formation of the embryo having two cells.

Successful fertilization is enabled by three processes, which also act as controls to ensure species-specificity. The first is that of chemotaxis which directs the movement of the sperm towards the ovum. Secondly there is an adhesive compatibility between the sperm and the egg. With the sperm adhered to the ovum, the third process of acrosomal reaction takes place; the front part of the spermatozoan head is capped by an acrosome which contains digestive enzymes to break down the zona pellucida and allow its entry. The entry of the sperm causes calcium to be released which blocks entry to other sperm cells. A parallel reaction takes place in the ovum called the zona reaction. This sees the release of cortical granules that release enzymes which digest sperm receptor proteins, thus preventing polyspermy. The granules also fuse with the plasma membrane and modify the zona pellucida in such a way as to prevent further sperm entry.

Cleavage

The beginning of the cleavage process is marked when the zygote divides through mitosis into two cells. This mitosis continues and the first two cells divide into four cells, then into eight cells and so on. Each division takes from 12 to 24 hours. The zygote is large compared to any other cell and undergoes cleavage without any overall increase in size. This means that with each successive subdivision, the ratio of nuclear to cytoplasmic material increases. Initially the dividing cells, called blastomeres (*blastos* Greek for sprout), are undifferentiated and aggregated into a sphere enclosed within the membrane of glycoproteins (termed the zona pellucida) of the ovum. When eight blastomeres have formed they begin to develop gap junctions, enabling them to develop in an integrated way and co-ordinate their response to physiological signals and environmental cues.

When the cells number around sixteen the solid sphere of cells within the zona pellucida is referred to as a **morula** At this stage the cells start to **bind** firmly together in a process called compaction, and cleavage continues as **cellular differentiation**.

Blastulation

Cleavage itself is the first stage in blastulation, the process of forming the blastocyst. Cells differentiate into an outer layer of cells (collectively called the trophoblast) and an inner cell mass. With further compaction the individual outer blastomeres, the trophoblasts, become indistinguishable. They are still enclosed within the zona pellucida. This compaction serves to make the structure watertight, containing the fluid that the cells will later secrete. The inner mass of cells differentiate to become embryoblasts and polarise at one end. They close together and form gap junctions, which facilitate cellular communication. This polarisation leaves a cavity, the blastocoel, creating a structure that is now termed the blastocyst. (In animals other than mammals, this is called the blastula.) The trophoblasts secrete fluid into the blastocoel. The resulting increase in size of the blastocyst causes it to hatch through the zona pellucida, which then disintegrates.

The inner cell mass will give rise to the pre-embryo, the amnion, yolk sac and allantois, while the fetal part of the placenta will form from the outer trophoblast layer. The embryo plus its membranes is called the concepts, and by this stage the concepts has reached the uterus. The zona pellucida ultimately disappears completely, and the now exposed cells of the trophoblast allow the blastocyst to attach itself to the endometrium, where it will implant. The formation of the hypoblast and epiblast, which are the two main layers of the bilaminar germ disc, occurs at the beginning of the second week. Either the embryoblast or the trophoblast will turn into two sub-layers. The inner cells will turn into the hypoblast layer, which will surround the other layer, called the epiblast, and these layers will form the embryonic disc that will develop into the embryo. The trophoblast will also develop two sub-layers: the cytotrophoblast, which is in front of the syncytiotrophoblast, which in turn lies within the endometrium.[9] Next, another layer called the exocoelomic membrane or Heuser's membrane will appear and surround the cytotrophoblast, as well as the primitive yolk sac. The syncytiotrophoblast will grow and will enter a phase called lacunar stage, in which some vacuoles will appear and be filled by blood in the following days. The development of the yolk sac starts with the hypoblastic flat cells that form the exocoelomic

membrane, which will coat the inner part of the cytotrophoblast to form the primitive yolk sac. An erosion of the endothelial lining of the maternal capillaries by the syncytiotrophoblastic cells of the sinusoids will form where the blood will begin to penetrate and flow through the trophoblast to give rise to the uteroplacental circulation. Subsequently new cells derived from yolk sac will be established between trophoblast and exocelomic membrane and will give rise to extra-embryonic mesoderm, which will form the chorionic cavity.

At the end of the second week of development, some cells of the trophoblast penetrate and form rounded columns into the syncytiotrophoblast. These columns are known as primary villi. At the same time, other migrating cells form into the exocelomic cavity a new cavity named the secondary or definitive yolk sac, smaller than the primitive yolk sac.

Implantation

After ovulation, the endometrial lining becomes transformed into a secretory lining in preparation of accepting the embryo. It becomes thickened, with its secretory glands becoming elongated, and is increasingly vascular. This lining of the uterine cavity (or womb) is now known as the decidua, and it produces a great number of large decidual cells in its increased interglandular tissue. The blastomeres in the blastocyst are arranged into an outer layer called the trophoblast.The trophoblast then differentiates into an inner layer, the cytotrophoblast, and an outer layer, the syncytiotrophoblast. The cytotrophoblast contains cuboidal epithelial cells and is the source of dividing cells, and the syncytiotrophoblast is a syncytial layer without cell boundaries.

The syncytiotrophoblast implants the blastocyst in the decidual epithelium by projections of chorionic villi, forming the embryonic part of the placenta. The placenta develops once the blastocyst is implanted, connecting the embryo to the uterine wall. The decidua here is termed the decidua basalis; it lies between the blastocyst and the myometrium and forms the maternal part of the placenta. The implantation is assisted by hydrolytic enzymes that erode the epithelium. The syncytiotrophoblast also produces human chorionic gonadotropin, a hormone that stimulates the release of progesterone from the corpus luteum. Progesterone enriches the uterus with a thick lining of blood vessels and capillaries so that it can oxygenate and sustain the developing embryo. The uterus liberates sugar from stored glycogen from its cells to nourish the embryo. The

villi begin to branch and contain blood vessels of the embryo. Other villi, called terminal or free villi, exchange nutrients. The embryo is joined to the trophoblastic shell by a narrow connecting stalk that develops into the umbilical cord to attach the placenta to the embryo. Arteries in the decidua are remodelled to increase the maternal blood flow into the intervillous spaces of the placenta, allowing gas exchange and the transfer of nutrients to the embryo. Waste products from the embryo will diffuse across the placenta.

As the syncytiotrophoblast starts to penetrate the uterine wall, the inner cell mass (embryoblast) also develops. The inner cell mass is the source of embryonic stem cells, which are pluripotent and can develop into any one of the three germ layer cells, and which have the potency to give rise to all the tissues and organs.

Embryonic disc

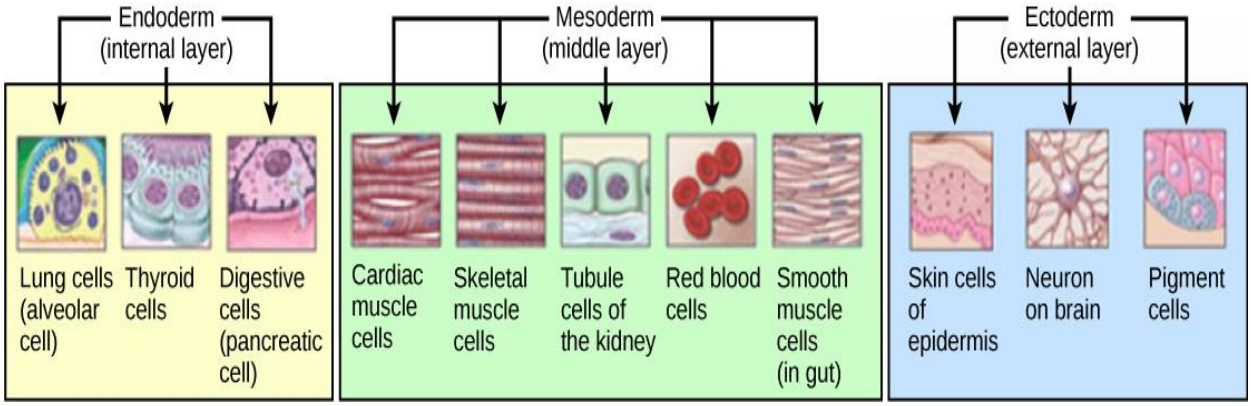
The embryoblast forms an embryonic disc, which is a bilaminar disc of two layers, an upper layer called the epiblast (primitive ectoderm) and a lower layer called the hypoblast (primitive endoderm). The disc is stretched between what will become the amniotic cavity and the yolk sac. The epiblast is adjacent to the trophoblast and made of columnar cells; the hypoblast is closest to the blastocyst cavity and made of cuboidal cells. The epiblast migrates away from the trophoblast downwards, forming the amniotic cavity, the lining of which is formed from amnioblasts developed from the epiblast. The hypoblast is pushed down and forms the yolk sac (exocoelomic cavity) lining. Some hypoblast cells migrate along the inner cytotrophoblast lining of the blastocoel, secreting an extracellular matrix along the way. These hypoblast cells and extracellular matrix are called Heuser's membrane (or the exocoelomic membrane), and they cover the blastocoel to form the yolk sac (or exocoelomic cavity). Cells of the hypoblast migrate along the outer edges of this reticulum and form the extraembryonic mesoderm; this disrupts the extraembryonic reticulum. Soon pockets form in the reticulum, which ultimately coalesce to form the chorionic cavity (extraembryonic coelom).

Gastrulation

The primitive streak, a linear band of cells formed by the migrating epiblast, appears, and this marks the beginning of gastrulation, which takes place around the seventeenth day (week 3) after fertilisation. The process of gastrulation reorganises the two-layer embryo

into a three-layer embryo, and also gives the embryo its specific head-to-tail, and front-to-back orientation, by way of the primitive streak which establishes bilateral symmetry. A primitive node (or primitive knot) forms in front of the primitive streak which is the organiser of neurulation.

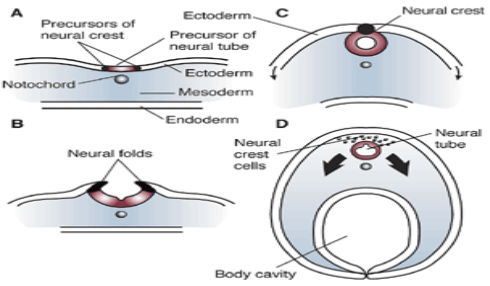
A primitive pit forms as a depression in the centre of the primitive node which connects to the notochord which lies directly underneath. The node has arisen from epiblasts of the amniotic cavity floor, and it is this node that induces the formation of the neural plate which serves as the basis for the nervous system. The neural plate will form opposite the primitive streak from ectodermal tissue which thickens and flattens into the neural plate. The epiblast in that region moves down into the streak at the location of the primitive pit where the process called ingression, which leads to the formation of the mesoderm takes place. This ingression sees the cells from the epiblast move into the primitive streak in an epithelial-mesenchymal transition; epithelial cells become mesenchymal stem cells, multipotent stromal cells that can differentiate into various cell types. The hypoblast is pushed out of the way and goes on to form the amnion. The epiblast keeps moving and forms a second layer, the mesoderm. The epiblast has now differentiated into the three germ layers of the embryo, so that the bilaminar disc is now a trilaminar disc, the gastrula.



The three germ layers are the ectoderm, mesoderm and endoderm, and are formed as three overlapping flat discs. It is from these three layers that all the structures and organs of

the body will be derived through the processes of somitogenesis, histogenesis and organogenesis. The embryonic endoderm is formed by invagination of epiblastic cells that migrate to the hypoblast, while the mesoderm is formed by the cells that develop between the epiblast and endoderm. In general, all germ layers will derive from the epiblast. The upper layer of ectoderm will give rise to the outermost layer of skin, central and peripheral nervous systems, eyes, inner ear, and many connective tissues.[16] The middle layer of mesoderm will give rise to the heart and the beginning of the circulatory system as well as the bones, muscles and kidneys. The inner layer of endoderm will serve as the starting point for the development of the lungs, intestine, thyroid, pancreas and bladder.

Neurulation



Following gastrulating, the ectoderm gives rise to epithelial and neural tissue, and the gastrula is now referred to as the neurula. The neural plate that has formed as a thickened plate from the ectoderm continues to broaden and its ends start to fold upwards as neural folds. Neurulation refers to this folding process whereby the neural plate is transformed into the neural tube, and this takes place during the fourth week. They fold, along a shallow neural groove which has formed as a dividing median line in the neural plate. This deepens as the folds continue to gain height, when they will meet and close together at the neural crest. The cells that migrate through the most cranial part of the primitive line form the paraxial mesoderm, which will give rise to the somitomeres that in the process of somitogenesis will differentiate into somites that will form the sclerotomes, the syndetomes, the myotomes andthe dermatomes toform cartilage and bone, tendons, derm is (skin), and muscle. The intermediate mesoderm gives rise to the urogenital tract and

consists of cells that migrate from the middle region of the primitive line. Other cells migrate through the caudal part of the primitive line and form the lateral mesoderm, and those cells migrating by the most caudal part contribute to the extraembryonic mesoderm.

The embryonic disc begins flat and round, but eventually elongates to have a wider cephalic part and narrow-shaped caudal end. At the beginning, the primitive line extends in cephalic direction and 18 days after fertilization returns caudally until it disappears. In the cephalic portion, the germ layer shows specific differentiation at the beginning of the 4th week, while in the caudal portion it occurs at the end of the 4th week. Cranial and caudal neuropores become progressively smaller until they close completely (by day 26) forming the neural tube.

Development of Organs and Organ System

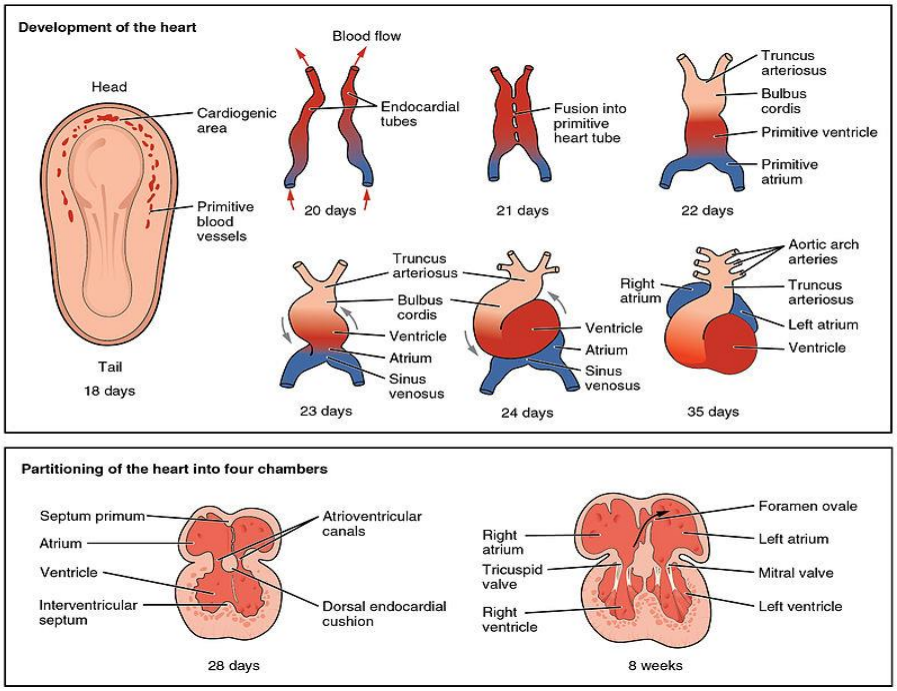
Organogenesis is the development of the organs that begins during the third to eighth week, and continues until birth. Sometimes full development, as in the lungs, continues after birth. Different organs take part in the development of the many organ systems of the body.

Blood

Haematopoietic stem cells that give rise to all the blood cells develop from the mesoderm. The development of blood formation takes place in clusters of blood cells, known as blood islands, in the yolk sac. Blood islands develop outside the embryo, on the umbilical vesicle, allantois, connecting stalk, and chorion, from mesodermal hemangioblasts.

In the centre of a blood island, hemangioblasts form the haematopoietic stem cells that are the precursor to all types of blood cell. In the periphery of a blood island the hemangioblasts differentiate into angioblasts the precursors to the blood vessels

Heart and circulatory system



The heart is the first functional organ to develop and starts to beat and pump blood at around 22 days. Cardiac myoblasts and blood islands in the splanchnopleuric mesenchyme on each side of the neural plate, give rise to the cardiogenic region. This is a horseshoe-shaped area near to the head of the embryo. By day 19, following cell signalling, two strands begin to form as tubes in this region, as a lumen develops within them. These two endocardial tubes grow and by day 21 have migrated towards each other and fused to form a single primitive heart tube, the tubular heart. This is enabled by the folding of the embryo which pushes the tubes into the thoracic cavity.

The endocardial tubes are forming, vasculogenesis (the development of the circulatory system) has begun. This starts on day 18 with cells in the splanchnopleuric mesoderm differentiating into angioblasts that develop into flattened endothelial cells. These join to form small vesicles called angiocysts which join up to form long vessels called angioblastic cords. These cords develop into a pervasive network of plexuses in the formation of the vascular network. This network grows by the additional budding and sprouting of new vessels in the process of angiogenesis. Following vasculogenesis and the development of an early vasculature, a stage of vascular remodelling takes place.

The tubular heart quickly forms five distinct regions. From head to tail, these are the infundibulum, bulbus cordis, primitive ventricle, primitive atrium, and the sinus venosus. Initially, all venous blood flows into the sinus venosus, and is propelled from tail to head to the truncus arteriosus. This will divide to form the aorta and pulmonary artery; the bulbus cordis will develop into the right (primitive) ventricle; the primitive ventricle will form the left ventricle; the primitive atrium will become the front parts of the left and right atria and their appendages, and the sinus venosus will develop into the posterior part of the right atrium, the sinoatrial node and the coronary sinus.

Cardiac looping begins to shape the heart as one of the processes of morphogenesis, and this completes by the end of the fourth week. Programmed cell death (apoptosis) at the joining surfaces enables fusion to take place.[21] In the middle of the fourth week, the sinus venosus receives blood from the three major veins: the vitelline, the umbilical and the common cardinal veins.

During the first two months of development, the interatrial septum begins to form. This septum divides the primitive atrium into a right and a left atrium. Firstly it starts as a crescent-shaped piece of tissue which grows downwards as the septum primum. The crescent shape prevents the complete closure of the atria allowing blood to be shunted from the right to the left atrium through the opening known as the ostium primum. This closes with further development of the system but before it does, a second opening (the ostium secundum) begins to form in the upper atrium enabling the continued shunting of blood.

A second septum (the septum secundum) begins to form to the right of the septum primum. This also leaves a small opening, the foramen ovale which is continuous with the previous opening of the ostium secundum. The septum primum is reduced to a small flap that acts as the valve of the foramen ovale and this remains until its closure at birth. Between the ventricles the septum inferius also forms which develops into the muscular interventricular septum

Digestive system

The digestive system starts to develop from the third week and by the twelfth week; the organs have correctly positioned themselves.

Respiratory system

The respiratory system develops from the lung bud, which appears in the ventral wall of the foregut about four weeks into development. The lung bud forms the trachea and two lateral growths known as the bronchial buds, which enlarge at the beginning of the fifth week to form the left and right main bronchi. These bronchi in turn form secondary (lobar) bronchi; three on the right and two on the left (reflecting the number of lung lobes). Tertiary bronchi form from secondary bronchi.

While the internal lining of the larynx originates from the lung bud, its cartilages and muscles originate from the fourth and sixth pharyngeal arches.

Urinary system

Kidneys

Three different kidney systems form in the developing embryo: the pronephros, the mesonephros and the metanephros. Only the metanephros develops into the permanent kidney. All three are derived from the intermediate mesoderm.

Pronephros

The pronephros derives from the intermediate mesoderm in the cervical region. It is not functional and degenerates before the end of the fourth week.

Mesonephros

The mesonephros derives from intermediate mesoderm in the upper thoracic to upper lumbar segments. Excretory tubules are formed and enter the mesonephric duct, which ends in the cloaca. The mesonephric duct atrophies in females, but participate in development of the reproductive system in males.

Metanephros

The metanephros appears in the fifth week of development. An outgrowth of the mesonephric duct, the ureteric bud, penetrates metanephric tissue to form the primitive renal pelvis, renal calyces and renal pyramids. The ureter is also formed.

Bladder and urethra

Between the fourth and seventh weeks of development, the urorectal septum divides the cloaca into the urogenital sinus and the anal canal. The upper part of the urogenital sinus forms the bladder, while the lower part forms the urethra.

Integumentary system

The superficial layer of the skin, the epidermis, is derived from the ectoderm. The deeper layer, the dermis, is derived from mesenchyme.

The formation of the epidermis begins in the second month of development and it acquires its definitive arrangement at the end of the fourth month. The ectoderm divides to form a flat layer of cells on the surface known as the periderm. Further division forms the individual layers of the epidermis.

The mesenchyme that will form the dermis is derived from three sources:

- The mesenchyme that forms the dermis in the limbs and body wall derives from the lateral plate mesoderm.
- The mesenchyme that forms the dermis in the back derives from paraxial mesoderm.
- The mesenchyme that forms the dermis in the face and neck derives from neural crest cells.

Nervous system

Late in the fourth week, the superior part of the neural tube bends ventrally as the cephalic flexure at the level of the future midbrain—the mesencephalon. Above the mesencephalon is the prosencephalon (future forebrain) and beneath it is the rhombencephalon (future hindbrain).

Cranial neural crest cells migrate to the pharyngeal arches as neural stem cells, where they develop in the process of neurogenesis into neurons.

The optical vesicle (which eventually becomes the optic nerve, retina and iris) forms at the basal plate of the prosencephalon. The alar plate of the prosencephalon expands to form the cerebral hemispheres (the telencephalon) whilst its basal plate becomes the diencephalon. Finally, the optic vesicle grows to form an optic outgrowth.

Development of Physical Features

Face and neck

From the third to the eighth week the face and neck develop.

Ears

The inner ear, middle ear and outer ear have distinct embryological origins.

Inner ear

At about 22 days into development, the ectoderm on each side of the rhombencephalon thickens to form otic placodes. These placodes invaginate to form otic pits, and then otic vesicles. The otic vesicles then form ventral and dorsal components.

The ventral component forms the saccule and the cochlear duct. In the sixth week of development the cochlear duct emerges and penetrates the surrounding mesenchyme, travelling in a spiral shape until it forms 2.5 turns by the end of the eighth week. The saccule is the remaining part of the ventral component. It remains connected to the cochlear duct via the narrow ductus reuniens.

The dorsal component forms the utricle and semicircular canals.

Middle ear

The tympanic cavity and eustachian tube are derived from the first pharyngeal pouch (a cavity lined by endoderm). The distal part of the cleft, the tubotympanic recess, widens to create the tympanic cavity. The proximal part of the cleft remains narrow and creates the eustachian tube.

The bones of the middle ear, the ossicles, derive from the cartilages of the pharyngeal arches. The malleus and incus derive from the cartilage of the first pharyngeal arch, whereas the stapes derives from the cartilage of the second pharyngeal arch.

Outer ear

The external auditory meatus develops from the dorsal portion of the first pharyngeal cleft. Six auricular hillocks, which are mesenchymal proliferations at the dorsal aspects of the first and second pharyngeal arches, form the auricle of the ear.

Eyes

The eyes begin to develop from the third week to the tenth week.

Limbs

At the end of the fourth week limb development begins. Limb buds appear on the ventrolateral aspect of the body. They consist of an outer layer of ectoderm and an inner part consisting of mesenchyme which is derived from the parietal layer of lateral plate mesoderm. Ectodermal cells at the distal end of the buds form the apical ectodermal ridge, which creates an area of rapidly proliferating mesenchymal cells known as the progress zone. Cartilage (some of which ultimately becomes bone) and muscle develop from the mesenchyme.

INVITRO FERTILIZATION

The first step in IVF is taking fertility medications for several months to help your ovaries produce several eggs that are mature and ready for fertilization. This is called ovulation induction. You may get regular ultrasounds or blood tests to measure your hormone levels and keep track of your egg production.

Once your ovaries have produced enough mature eggs, your doctor removes the eggs from your body (this is called egg retrieval). Egg retrieval is a minor surgical procedure that's done at your doctor's office or at a fertility clinic.

You'll get medicine to help you be relaxed and comfortable during the procedure. Using an ultrasound to see inside your body, the doctor puts a thin, hollow tube through your vagina and into the ovary and follicles that hold your eggs. The needle is connected to a suction device that gently pulls the eggs out of each follicle.

In a lab, your eggs are mixed with sperm cells from your partner or a donor — this is called insemination. The eggs and sperm are stored together in a special container, and fertilization happens. For sperm that have lower motility (don't swim as well), they may be injected directly into the eggs to promote fertilization. As the cells in the fertilized eggs divide and become embryos, people who work at the lab monitor the progress.

About 3-5 days after the egg retrieval, 1 or more embryos are put into your uterus (this is called embryo transfer). The doctor slides a thin tube through your cervix into your uterus, and inserts the embryo directly into your uterus through the tube.

Pregnancy happens if any of the embryos attach to the lining of your uterus. Embryo transfer is done at your doctor's office or at a fertility clinic, and it's usually not painful.

Plan on resting for the rest of the day after your embryo transfer. You can go back to your normal activities the next day. You may also take pills or get daily shots of a hormone called progesterone for the first 8-10 weeks after the embryo transfer. The hormones make it easier for the embryo to survive in your uterus.

Why In Vitro Fertilization?

In vitro fertilization (IVF) is a treatment for infertility or certain genetic problems. Sometimes, IVF is offered as an essential treatment for infertility for women over 40 years. Here are some examples of the other health conditions it could be used to treat:

- Fallopian tube damage: the fallopian tube damage or blockage makes fertilizing an egg seems like an impossible mission, as it prevents the embryo from traveling and implanting on the uterus to develop later.
- Uterine fibroids where there are tumors in the wall of the uterus and common among women in their 30s and 40s.
- Ovulation problems or disorders: the more infrequent, the fewer eggs available for fertilization.
- Previous tubal sterilization, if the woman has had any kind of sterilization where the fallopian tubes are removed or cut that could prevent pregnancy forever. IVF could be a good alternative to tubal ligation reversal.
- Unexplained infertility means that there is no obvious explanation for infertility.
- Endometriosis takes place when the uterine tissue implants and develops outside of the uterus that leads to negative effects on the function of the uterus, ovaries and fallopian tubes.

- Genetic disorders; if one or both of the partners are at risk of giving a genetic disorder to the child. After the eggs are collected and fertilized, they got screened to check if there is a certain genetic problem or not.
- Impaired sperm function. Weak moving sperm (poor mobility) or any abnormalities in sperm size can make fertilizing an egg is a difficult task.

In Vitro Fertilization And Embryo Transfer

IVFET can be used to overcome infertility caused by numerous conditions including tubal disease, endometriosis and oligospermia. A first step in IVFET is to prepare the woman for removal of eggs (oocytes). Two methods are used to accomplish this. Sometimes oocytes can be obtained during a natural cycle of a woman by determining the time of the marked increase in the luteinizing hormone level in the blood, which precedes ovulation by about 1 1/2 days. Using a natural cycle, however, frequent blood samples must be analyzed to exactly pinpoint the increase in this hormone level. Only one mature egg is usually by this method. Alternatively, follicular growth and maturation, which leads to ovulation, can be induced by the use of various fertility drugs such as human menopausal gonadotrophin. The subsequent development of ovarian follicles can be monitored by ultrasound and by measuring blood estrogen levels. By this method, which is most commonly used today, more than one oocyte is stimulated to develop and can be obtained for fertilization.

Just before the timed ovulation would occur, oocytes are removed from the ovary either laparoscopy or by needle aspiration guided by ultrasonography. The eggs, with their adherent nurse cells, are placed in a petri dish so that their state of maturation can be assessed using the state of dispersion of the attached cells as a marker. Fertilization of the mature egg is accomplished by incubation for approximately 24 hours in the petri dish with washed sperm that have been treated to ensure capacitation. Fertilization is defined by the visible presence of two pronuclei in the newly formed zygote.

The first cleavage of the zygote occurs approximately 1 1/2 days after insemination. A catheter is used to transfer the dividing embryo into the lumen of the uterus at sore between the 2- and 16-cell stage. To supplement the natural luteal phase, hormones such as progesterone are sometimes administered after transfer of the embryo, (or embryos if more than one oocyte has been fertilized) to the uterus. Pregnancy is established when the

developing embryo implants itself into the wall of the uterus. Implantation can be documented by a measured increase in blood levels of human chorionic gonadotrophin.

Sometimes, a greater number of mature eggs are harvested than can usefully be implanted. Increasingly, these excess eggs are fertilized and preserved by cryopreservation for subsequent use.

Birth control methods

Birth control (contraception) is any method, medicine, or device used to prevent pregnancy. Women can choose from many different types of birth control. Some work better than others at preventing pregnancy. The type of birth control you use depends on your health, your desire to have children now or in the future, and your need to prevent sexually transmitted infections. Your doctor can help you decide which type is best for you right now.

What are the different types of birth control?

Women can choose from many different types of birth control methods. These include, in order of most effective to least effective at preventing pregnancy:

- **Female and male sterilization** (female tubal ligation or occlusion, male vasectomy) — Birth control that prevents pregnancy for the rest of your life through surgery or a medical procedure.
- *Long-acting* **reversible contraceptives or "LARC" methods** (intrauterine devices, hormonal implants) — Birth control your doctor inserts one time and you do not have to remember to use birth control every day or month. LARCs last for 3 to 10 years, depending on the method.
- *Short-acting* **hormonal methods** (pill, mini pills, patch, shot, vaginal ring) — Birth control your doctor prescribes that you remember to take every day or month. The shot requires you to get a shot from your doctor every 3 months.
- **Barrier methods** (condoms, diaphragms, sponge, cervical cap) — Birth control you use each time you have sex.
- **Natural rhythm methods** — Not using a type of birth control but instead avoiding sex and/or using birth control only on the days when you are most fertile (most likely to get

pregnant). An ovulation home test kit or a fertility monitor can help you find your most fertile days.

Rh factor and its Significance

An antigen found on the surface of red blood cells. Red blood cells with the antigen are said to be Rh positive (Rh+). Those without the surface antigen are said to be Rh negative (Rh-). Blood used in transfusions much match donors for Rh status as well as for ABO blood group, as Rh- patients will develop anemia if given R+ blood. Rh typing is also important during abortion, miscarriage, pregnancy, and birth, as mother and fetus may not be Rh-compatible. Rh stands for rhesus monkeys, in whose blood this antigen was first found.

Rh factor is important only during a pregnancy in which an Rh negative woman is carrying a fetus who might be Rh positive. This can occur when an Rh negative woman conceives a baby with an Rh positive man. The gene for Rh positive blood is dominant over the gene for Rh negative blood, so their baby will be Rh positive. If the Rh positive father also carries the gene for Rh negative blood, his babies have a 50% chance of inheriting Rh negative blood and a 50% chance of inheriting Rh positive blood. If both parents are Rh negative, their babies will always be Rh negative. In order to protect their future babies from Rh disease, all women of childbearing age should know their Rh status before becoming pregnant.

Significance

Rhesus (Rh) factor is an inherited protein found on the surface of red blood cells. If your blood has the protein, you're Rh positive. If your blood lacks the protein, you're Rh negative. Rh positive is the most common blood type

- D antigen, after A and B, is the most important RBC antigen in transfusion practice.
- Individuals who lack D antigen DO NOT have anti-D.Antibody produced through exposure to D antigen through transfusion or pregnancy.Immunogenicity of D greater than that of all other RBC antigens studied.Has been reported that 80%> of D neg

individuals who receive single unit of D pos blood can be expected to develop immune anti-D.Testing for D is routinely performed so D neg will be transfused with D neg.

- When the mother is Rh- and the father is Rh+, either 50 or 100 per cent of the offspring will be Rh positive, the difference in percentage being dependent on whether the Rh factor in father is homo- or heterozygous. If fetal blood containing the Rh factor crosses the placental barrier and gains access to the maternal circulation, agglutinins may be produced in her blood.
- If agglutinins are produced either as a result of direct intentional transfusion or by occult transfusion from the fetus, the subsequent introduction of large amounts of blood containing the Rh antigen will result in the agglutination of this newly introduced blood and a fatal transfusion reaction may occur.
- When it becomes necessary to transfuse an infant suffering from erythroblastosis, the mother's blood should never be used. If, as we believe, the disease is due to the effect on the fetus of agglutinins transmitted to it from the maternal circulation, further introduction of maternal blood would result in the introduction of more agglutinins which would aggravate the disease.
- It has been contended that Rh negative blood should always be used to transfuse these infants because, in spite of the fact that they are practically always Rh positive, there is a possibility of free anti Rh agglutinins being present in their blood stream. We have not observed such agglutinins and have been unable to find a record of their demonstration. It may be questioned, therefore, whether it is necessary to transfuse with Rh negative blood on this basis.
- If cells and serum of patient and potential donor show no agglutination after incubation at 37°C. for one hour followed by centrifugation at 600 revolutions for one minute, the blood of this donor can be used with safety, regardless of whether it is Rh positive or Rh negative.
- Since the majority of women who give birth to babies with erythroblastosis are known to be Rh negative and may show anti-Rh agglutinins, it is essential to use blood from a known Rh negative donor if it becomes necessary to transfuse one of these women.
- Since the Rh factor is present in approximately 86 per cent of the general population, about 12 per cent of all marriages will be between couples where the wife is Rh

negative, and the husband Rh positive. It is in this group that the wife is capable of becoming sensitized to the Rh factor and of subsequently reacting on the fetus to produce erythroblastosis. Erythroblastosis, however, occurs in only a small percentage of these women and in our experience has been found in only about 0.1 per cent of all pregnancies (The Chicago Lying-in Hospital). To account for the difference between potential and actual incidence, there are several conditions which may contribute: (1) in childless or one-child marriages the limitation in the number of offspring makes the production of erythroblastosis impossible, (2) the Rh antigen in the infant may vary in its ability to stimulate the production of agglutinins in the maternal blood, (3) the ability of the placenta to prevent the passage of the Rh antigen may vary, (4) the maternal response to the introduction of the Rh antigen into the blood stream may vary, (5) the ability of the placenta to permit passage of agglutinins may vary.

- It becomes apparent that although a fundamental incompatibility between the genetic constitution of the male and female germ cells may create a situation in which the occurrence of erythroblastosis becomes a possibility, there must be other superimposed factors which determine whether or not the possibility will be realized.
- A few women giving birth to babies who appear to suffer from erythroblastosis are Rh positive and a few infants suffering from the disease are Rh negative. It may be possible that these infants are actually suffering from a different disease entity. It is certain that severe jaundice or generalized edema can occur independently of erythroblastosis, and it is possible that in a small proportion of those infants who have the fundamental disturbance in the formation and destruction of erythrocytes which is usually characteristic of erythroblastosis, the condition may be a response to an ontirely different etiologic agent. Only further investigations can settle this point.
- It seems justifiable, however, to conclude that in any case where the diagnosis of erythroblastosis is doubtful, support for the diagnosis is obtained by finding the maternal blood Rh negative and the paternal and infant blood Rh positive. If the mother is Rh positive, the diagnosis of erythroblastosis is less probable.

Questions:

Section A : 1.Manchette 2.Acrosome 3.chemotaxis 4. Neurulation

5. Neurogenesis 6. IVF

Section B : Write a short note on Developmental biology and Human welfare.

Describe the structure of Sperm with suitable diagram

Explain the Structure of Ovum

Describe the Neurulation Process.

Write about the Significance of Rh factor

Section C : Describe the Development of Human embryo up to Child Birth.

Explain Briefly about Invitro Fertilization and Embryo Transfer.

Describe the birth Control Methods

IMMONOLOGY

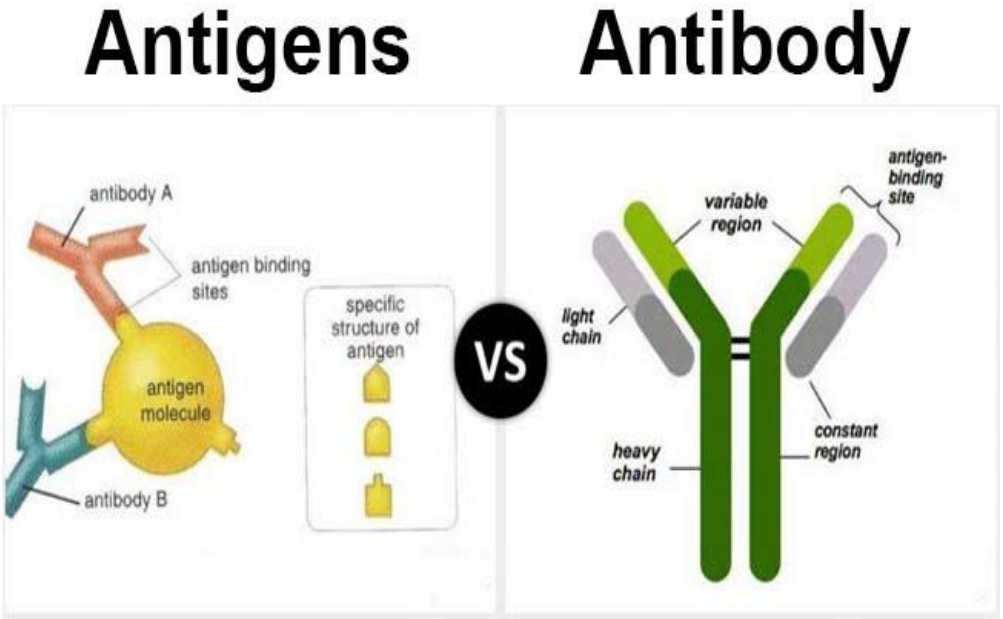
Unit IV

ANTIGEN – ANTIBODY REACTION

Structure of Antigens

The term antigen is derived from antibody generation, referring to any substance that is capable of eliciting an immune response (e.g., the production of specific antibody molecules). Generally, antigens are foreign proteins or their fragments that enter host body via an infection. However, in some cases, the body's own proteins may act as antigens and induce an autoimmune response. Bacteria and viruses contain antigens, either on their surface, or inside. These antigens can be isolated and used to develop vaccines.

Antigens are generally of high molecular weight, and commonly are proteins or polysaccharides. Polypeptides, lipids, nucleic acids, and many other materials can also function as antigens. Immune responses may also be generated against smaller substances, called haptens, if these are chemically coupled to a larger carrier protein, such as bovine serum albumin, keyhole limpet hemocyanin (KLH), or other synthetic matrices.



A variety of molecules such as drugs, simple sugars, amino acids, small peptides, phospholipids, or triglycerides may function as haptens. Thus, given enough time, just about any foreign substance will be identified by the immune system and evoke specific antibody production. However, this specific immune response is highly variable and depends much in part on the size, structure, and composition of antigens.

Structure of antibodies

An antibody is defined as “an immunoglobulin capable of specific combination with the antigen that caused its production in a susceptible animal.” Antibodies are produced in response to the invasion of foreign molecules in the body. An antibody, abbreviated as Ab, is commonly referred to as an immunoglobulin or Ig. Human immunoglobulins are a group of structurally and functionally similar glycoproteins (82-96% protein and 4-18% carbohydrate) that confer humoral immunity.

Structure

Antibodies exist as one or more copies of a Y-shaped unit, composed of four polypeptide chains. Each Y contains two identical copies of a heavy chain and two identical copies of a light chain, named as such by their relative molecular weights. This Y-shaped unit is composed of the two variable, antigen-specific F(ab) arms, which are critical for actual antigen binding, and the constant Fc “tail” that binds immune cell Fc receptors and also serves as a useful “handle” for manipulating the antibody during most immunochemical procedures. The number of F(ab) regions on the antibody corresponds with its subclass (see below), and determines the valency of the antibody (loosely stated, the number of “arms” with which the antibody may bind its antigen).

Antibodies can be divided into five classes: IgG, IgM, IgA, IgD, and IgE, based on the number of Y units and the type of heavy chain. Heavy chains of IgG, IgM, IgA, IgD, and IgE, are known as g, μ, a, d, and e, respectively. The light chains of any antibody can be classified as either a kappa (κ) or lambda (λ) type (based on small polypeptide structural differences); however, the heavy chain determines the subclass of each antibody.

The subclasses of antibodies differ in the number of disulfide bonds and the length of the hinge region. The most commonly used antibody in immunochemical procedures is of the IgG class because this is the major immunoglobulin class released in serum.

- **IgA:** In the blood IgA are present in low levels in monomeric form. They are most active at mucosal surfaces where they are present in dimeric form and provide the primary defense at mucosal surfaces. More IgA is produced in mucosal linings than all other types of antibody combined. Its major function is to act as a neutralizing antibody. High levels of IgA are present in saliva, tears, and breast milk. In humans two IgA subtypes are known to exist whereas in mice only one form is reported. IgA1 may account up to 85% of the total IgA in serum. Selective IgA deficiency is

one of the most common immunodeficiency diseases that increases susceptibility to infections. IgA deficiencies are commonly seen in patients with autoimmune diseases and allergic disorders. IgA has a half-life of about 5 days.

- **IgD:** It is a monomeric antibody with two epitope binding sites and is found on the surface of most B lymphocytes. Its precise function is still disputed, but is suggested to acts as an antigen receptor required for B cell activation. IgD is also reported to bind to basophils and mast cells and activate them to produce antimicrobial factors. It's also believed to play a role in eliminating B-lymphocytes that produce self-reactive autoantibodies. IgD is also produced in a secreted form that is found in serum in small quantities and contains two heavy chains of the δ class and two light chains. IgD has a half life of about 3 days.
- **IgE:** This group of antibodies is effective at mucosal surfaces, blood, and tissues. It is present as monomer consisting of two heavy chains (ε chain) and two light chains. The ε chain contains 4 Ig-like constant domains. In serum, it is present in low concentrations contributing to only about 0.002% of total serum antibodies. Most IgE is tightly bound to its receptors on mast cells and basophils via the Fc region. It plays a crucial role in hypersensitivity reactions and its production is strictly controlled by cytokines. IgE has a half-life of about 2 days.
- **IgG:** This is the most abundant class of antibodies in the blood, comprising up to 80% of the total serum antibodies. It is present in monomeric form. Four subclasses of IgG have been described depending on their abundance (IgG1>IgG2>IgG3>IgG4) and the subclass produced is dependent on the type of cytokine present.
- IgG1 and IgG3 exhibit high affinity for Fc receptors on phagocytes, while IgG2 exhibits very low affinity and IgG4 has moderate affinity for Fc receptors IgGs are capable of exiting the circulatory system and enter tissues. IgG1, IgG3, and IgG4 can cross placental barrier to provide protection for newborns. IgGs are efficient at activating the complement system, and are very effective for opsonization using Fc receptors on phagocytes. Through its Fc region IgG can also bind to natural killer cells and participate in antibody-dependent cytotoxicity. IgG has a half-life ranging from 7 to 23 days, depending on its subclass.
- **IgM:** This class of immunoglobulin is first to be produced in response to infection and is found either on membranes of B cells or as a 5-subunit macromolecule

secreted by plasma cells. It is also the first immunoglobulin class to be synthesized by the neonates. The surface IgM differs from the secreted form in its Fc region. Surface IgM binds directly as an integral membrane protein and not to the IgM Fc receptor. Secreted IgM is a pentameric molecule where multiple immunoglobulins are covalently linked with disulfide bonds. This structure provides multiple binding sites. Each monomer consists of two light chains (either κ or λ) and two heavy chains. Because of its pentameric nature IgM is particularly suited for activating complement and causing agglutination. IgM has a half-life of about 5 days.

BINDING SITES OF Ig – Ab

The interactions between antigens and antibodies are known as ***antigen–antibody reactions***. The reactions are highly specific, and an antigen reacts only with antibodies produced by it or with closely related antigens. Antibodies recognize molecular shapes (epitopes) on antigens. Generally, the better the fit of the epitope (in terms of geometry and chemical character) to the antibody combining site, the more favorable the interactions that will be formed between the antibody and antigen and the higher the affinity of the antibody for antigen.

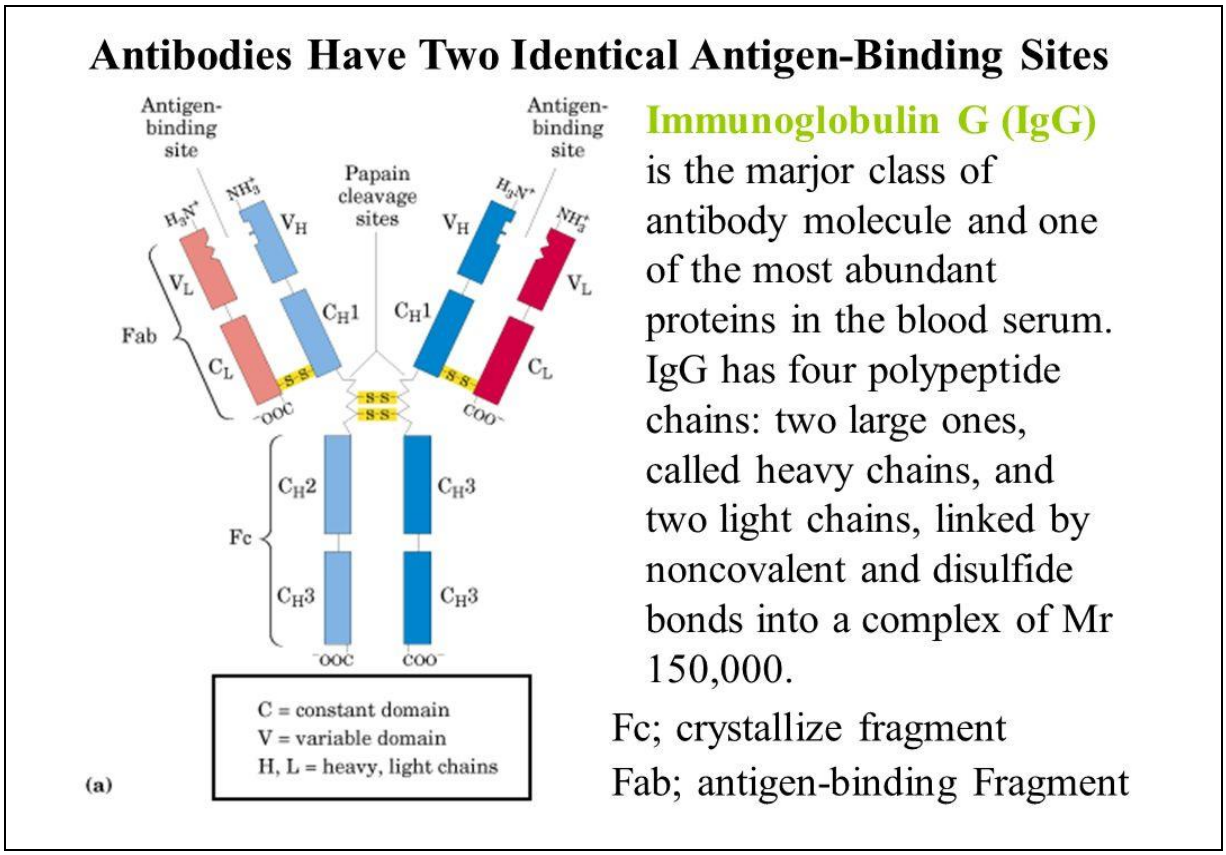
The antigen- antibody interaction is bimolecular irreversible association between antigen and antibody. The association between antigen and antibody includes various non-covalent interactions between epitope (antigenic determinant) and variable region (V_H/V_L) domain of antibody.

Chemical Bonds Responsible for the Antigen–Antibody Reaction

The interaction between the Ab-binding site and the epitope involves exclusively non-covalent bonds, in a similar manner to that in which proteins bind to their cellular receptors, or enzymes bind to their substrates. The binding is reversible and can be prevented or dissociated by high ionic strength or extreme pH. The following intermolecular forces are involved in Ag–Ab binding:

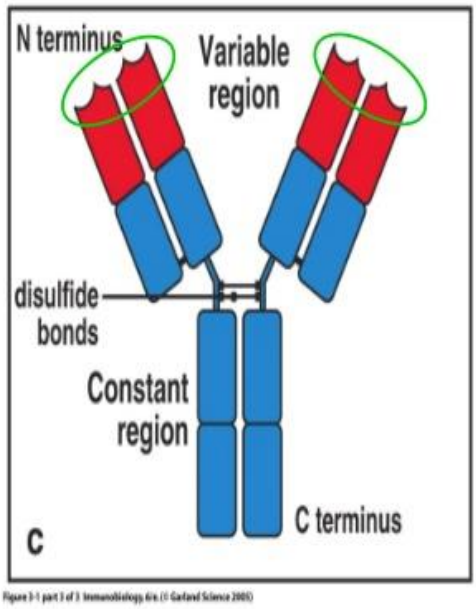
1. **Electrostatic bonds:** This result from the attraction between oppositely charged ionic groups of two protein side chains; for example, an ionized amino group (NH_4^+) on a lysine in the Ab, and an ionized carboxyl group (COO^-) on an aspartate residue in the Ag.

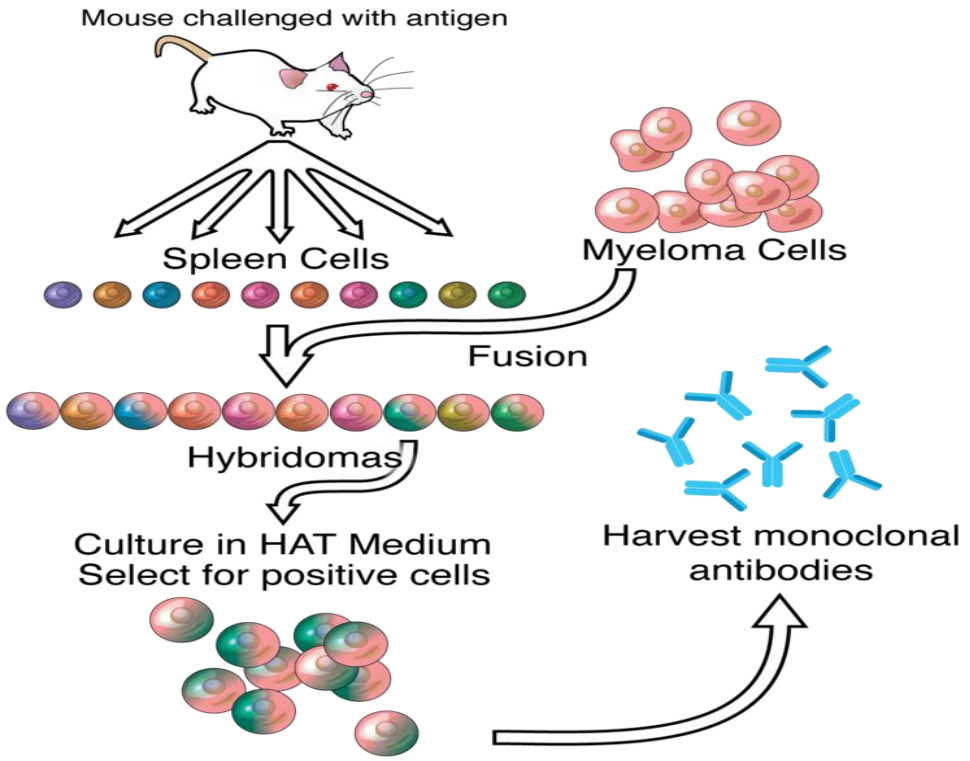
2. **Hydrogen bonding**: When the Ag and Ab are in very close proximity, relatively weak hydrogen bonds can be formed between hydrophilic groups (e.g., OH and C=O, NH and C=O, and NH and OH groups).
3. **Hydrophobic interactions**: Hydrophobic groups, such as the side chains of valine, leucine, and phenylalanine, tend to associate due to Van der Waals bonding and coalesce in an aqueous environment, excluding water molecules from their surroundings. As a consequence, the distance between them decreases, enhancing the energies of attraction involved. This type of interaction is estimated to contribute up to 50% of the total strength of the Ag–Ab bond.
4. **Van der Waals bonds:** These forces depend upon interactions between the "electron clouds" that surround the Ag and Ab molecules. The interaction has been compared to that which might exist between alternating dipoles in two molecules, alternating in such a way that, at any given moment, oppositely oriented dipoles will be present in closely apposed areas of the Ag and Ab molecules.



Antigen-binding site

- Hypervariable regions (HV regions)
  - Small regions of high amino-acid sequence diversity within the variable regions of immunoglobulin and T-cell receptor.
  - They correspond to the complementarity-determining regions.





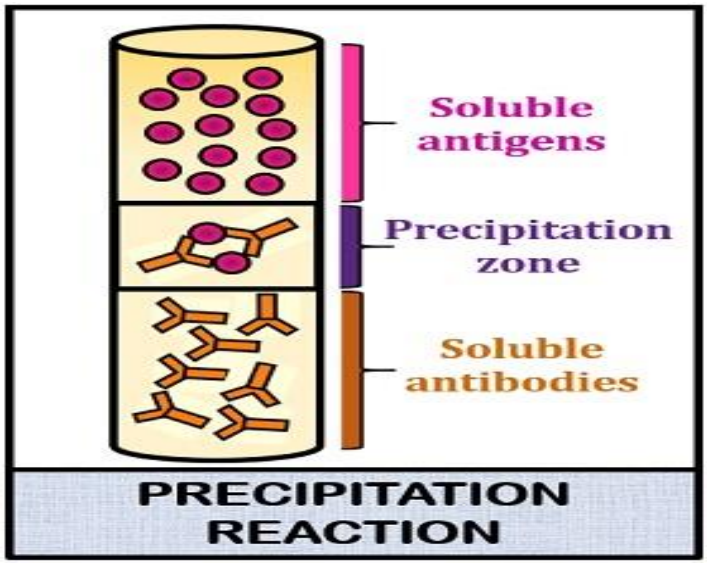
TYPES OF AG-AB REACTIONS

PRECIPITATION

It is a type of antigen-antibody reaction, in which the antigen occurs in a soluble form. When a soluble antigen reacts with its specific antibody, at an optimum temperature and P^H in the presence of electrolyte antigen-antibody complex forms insoluble precipitate. This reaction is called a precipitation reaction. A lattice is formed between the antigens and antibodies; in certain cases, it is visible as an insoluble precipitate. Antibodies that aggregate soluble antigens are called **precipitins.**

The interaction of antibody with soluble antigen may cause the formation of insoluble lattice that will precipitate out of solution. Formation of an antigen-antibody lattice depends on the valency of both the antibody and antigen. The antibody must be **bivalent**; a precipitate will not form with monovalent Fab fragments. The antigen must be **bivalent or polyvalent**; that is it must have at least two copies of same epitope or different epitopes that react with different antibodies present in polyclonal sera. Antigen and antibody must be in an appropriate concentration relative to each other.

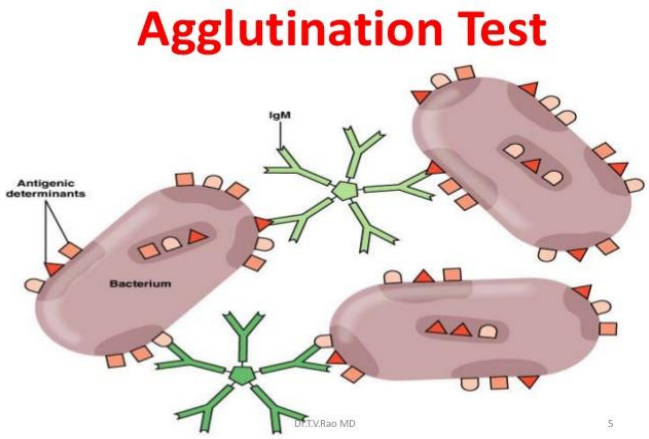
1. Antigen access: Too much antigen prevents efficient crosslinking/lattice formation.
2. Antibody access: Too much antibody prevents efficient crosslinking/lattice formation.
3. Equivalent Antigen and Antibody: Maximum amount of lattice (Precipitate) is formed



AGGLUTINATION

Agglutination is an antigen-antibody reaction in which a particulate antigen combines with its antibody in the presence of electrolytes at a specified temperature and pH resulting in the formation of visible clumping of particles. It occurs optimally when antigens and antibodies react in equivalent proportions. This reaction is analogous to the precipitation reaction in that antibodies act as a bridge to form a lattice network of antibodies and the cells that carry the antigen on their surface. Because cells are so much larger than a soluble antigen, the result is more visible when the cells aggregate into clumps.

When particulate antigens react with specific antibody, antigen-antibody complex forms visible clumping under optimum P^H and temperature. Such a reaction is called agglutination. Antibodies that produce such reactions are called **agglutinins**.



OPSONIZATION

The term opsonization refers to the capacity of antibodies and complement components (as well as other proteins) to coat dangerous antigens that can then be recognized by antibodies or complement receptors on phagocytic cells.

Opsonization is the molecular mechanism whereby molecules, microbes, or apoptotic cells are chemically modified to have stronger interactions with cell surface receptors on phagocytes and antibodies.

This is the mechanism of identifying invading particles (antigens) by the use of specific components called opsonins. The opsonins act as markers or tags that allow recognition by the immune system of the body.

An opsonin is any molecule that enhances phagocytosis by marking an antigen for an immune response or marking dead cells for recycling.

The purpose of opsonization is to make the antigens palatable to the antibody or the phagocytic cells. Opsonization of pathogens can occur via antibodies or the complement system.

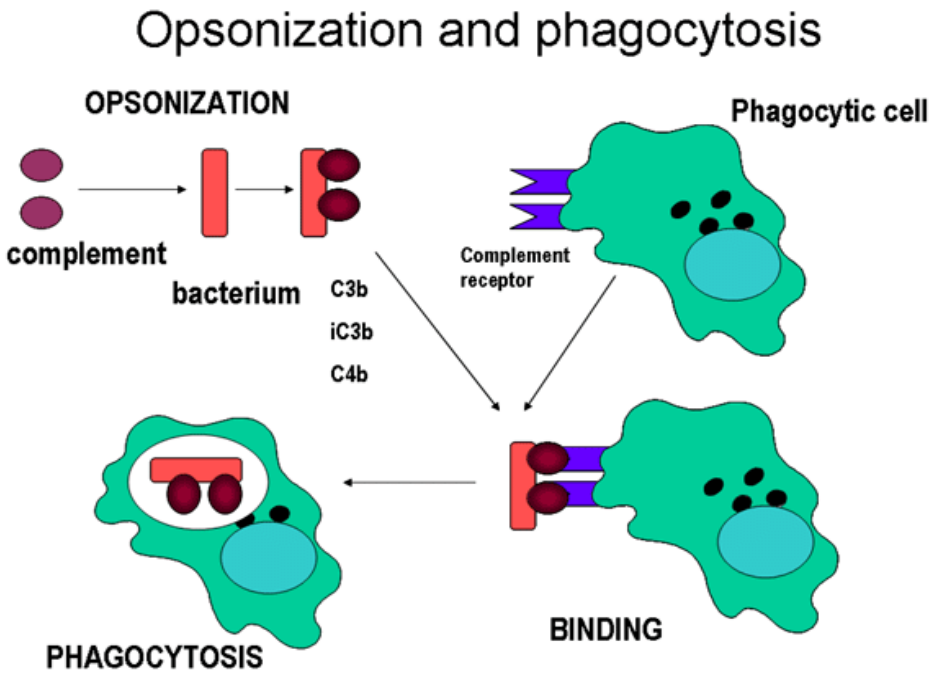
CYTOLYSIS

These are antibodies, which leads to lysis (destruction) of the antigens, which are called a lytic antibody. This cytolysis is complement dependant where complement is activated and leading to lysis of antigens e.g. hemolysis of RBC. Ag & Ab complex is recognized by complement and there is classical complement pathway activation, making holes formation in the cell membrane and leading to osmotic death.

The antigen-antibody reaction leads tri lysis of bacterial cell walls or RBCs is valuable procedures in the serological testing. Lysis is visible disintegration showing that antigen and antibody have reacted.

FLOCCULATION

Flocculation tests are designed for antibody detection and are based on the interaction of soluble antigens with antibodies, producing a precipitate of fine particles that can be seen with the naked eye. Direct bacterial agglutination uses whole pathogens as a source of antigen.

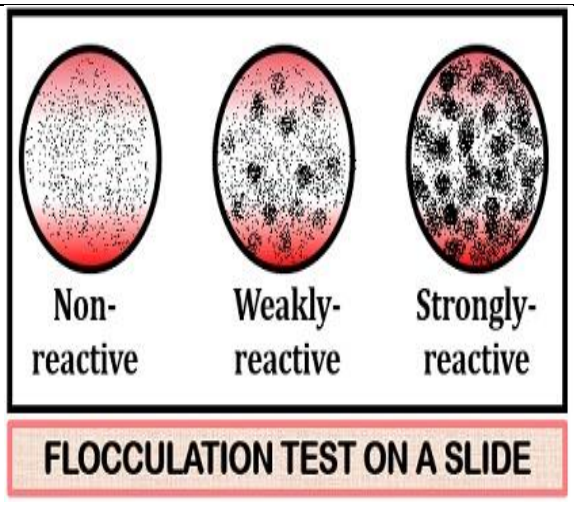


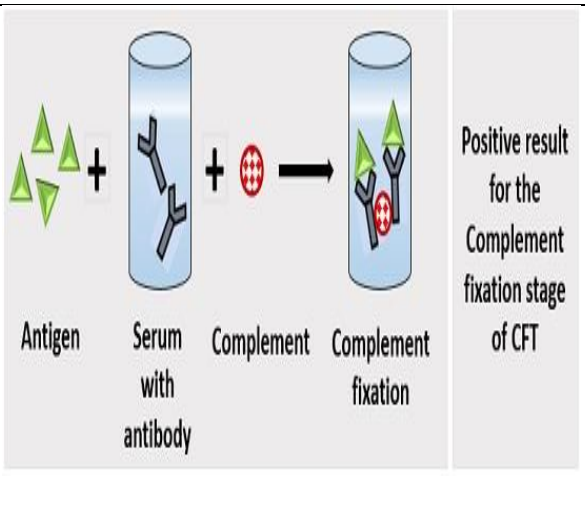
COMPLEMENT FIXATION

Complement fixation is a classic method for demonstrating the presence of antibody in patient serum. The complement fixation test consists of two components. The first component is an indicator system that uses combination of sheep red blood cells, complement-fixing antibody such as immunoglobulin G produced against the sheep red blood cells and an exogenous source of complement usually guinea pig serum.

The second component is a known antigen and patient serum added to a suspension of sheep red blood cells in addition to complement. These two components of the complement fixation method are tested in sequence. Patient serum is first added to the known antigen, and complement is added to the solution. If the serum contains antibody to the antigen, the resulting antigen-antibody complexes will bind all of the complement. Sheep red blood cells and the anti-sheep antibody are then added.

If complement has not been bound by an antigen-antibody complex formed from the patient serum and known antigens, it is available to bind to the indicator system of sheep cells and anti-sheep antibody. Lysis of the indicator sheep red blood cells signifies both a lack of antibody in patient serum and a negative complement fixation test. If the patient's serum does contain a complement-fixing antibody, a positive result will be indicated by the lack of red blood cell lysis.





HISTOCOMPATIBILITY COMPLEX (MHC) - TYPES AND IMPORTANCE

- Major histocompatibility complex (MHC) is the cluster of gene arranged within a long continuous stretch of DNA on chromosome number 6 in Human which encodes MHC molecules.
- MHC molecule is a cell surface glycoprotein receptor present in APCs and acts as antigen presenting structure It plays vital role in immune recognition, including interaction between T cells and other cell types.
- In Human MHC is known as Human Leucocyte antigen (HLA) complex and the genes of MHC are recognized in three classes, consequently there are three types of MHC molecules.
  - **Class I MHC**
  - **Class II MHC**
  - **Class III MHC**

Major Histocompatibility Complex Class I (MHC-I)

Class I molecules consist of a heavy polypeptide chain of 44 kDa non-covalently linked to a smaller 12 kDa peptide called β_2-microglobulin. The largest part of the heavy chain is organized into three globular domains (α_1, α_2 and α_3) which protrude from the cell surface; a hydrophobic section anchors the molecule in the membrane and a short hydrophilic sequence carries the C-terminus into the cytoplasm. The heavy chain has a variable and constant region. The variable region is highly pleomorphic. The polymorphism of these molecules is important in the recognition of self and non-self. The constant region of the heavy chain binds with the CD8 proteins of the cytotoxic T cells.

X-ray analysis of crystals of a human class I molecule shows both β_2 -microglobulin and the α_3 region resemble classic Ig domains in their folding pattern. The α_1 and α_2 domains interact to form a platform of eight antiparallel β strands spanned by two long α-helical regions. The structure forms a deep groove, or cleft with the long α helices as sides and the β strands of the β sheet as the bottom. This **peptide-binding groove** is located on the top surface of the class I MHC molecule, and bind a peptide of 8 to 10 amino acids.

Class I proteins are involved in **graft rejection and cell-mediated cytolysis**.

Major Histocompatibility Complex Class II (MHC-II)

Class II MHC molecules are also transmembrane glycoproteins, consisting of α and β polypeptide chains of molecular weight 33-kDa α chain and a 28-kDa β chain, which associate by noncovalent interactions. Class II molecule contains two external domains: α_1 and α_2 domains in one chain and β_1 and $\beta_2$domains in the other. There is considerable sequence homology with class I.

Structural studies have shown that the α_2 and β_2 domains, the ones nearest to the cell membrane assume the characteristic Ig fold, while the α_1 and β_1 domains form the peptide-binding groove for processed antigen. The peptide-binding groove of class II molecules is composed of a floor of eight antiparallel β strands and sides of antiparallel α helices, where peptides typically ranging from 13 to 18 amino acids can bind.

Class II proteins are primarily responsible for the **graft-versus-host response and the mixed leukocyte response.**

MHC class-III:

- MHc-III are diverse group of molecules that serves a wide variety of functions in immune system.
- MHC-III are not a marker on cell surface.

Functions of MHC class-III:

- Involved in complement activation
- Involved in inflammation caused by cytokines, tumor necrosis factors etc

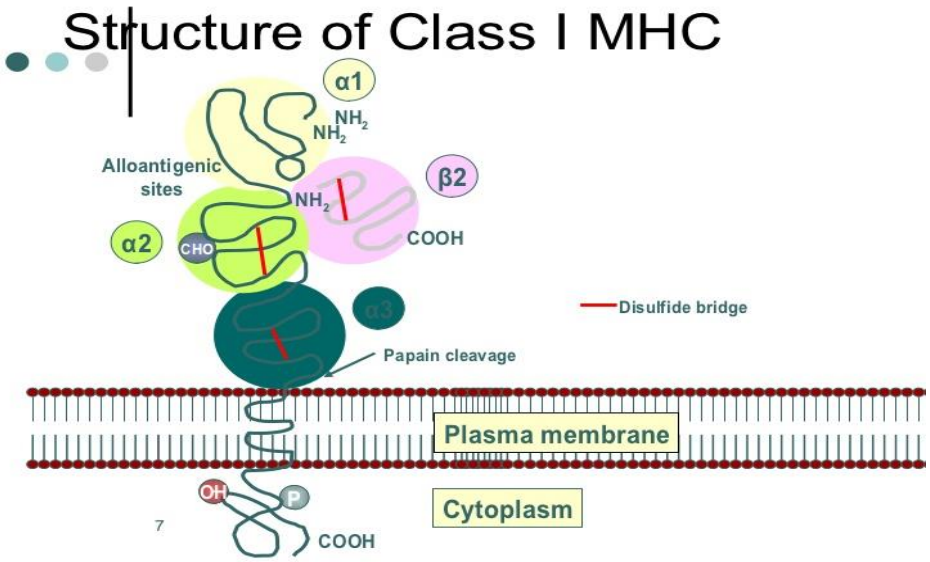
Distribution of MHC

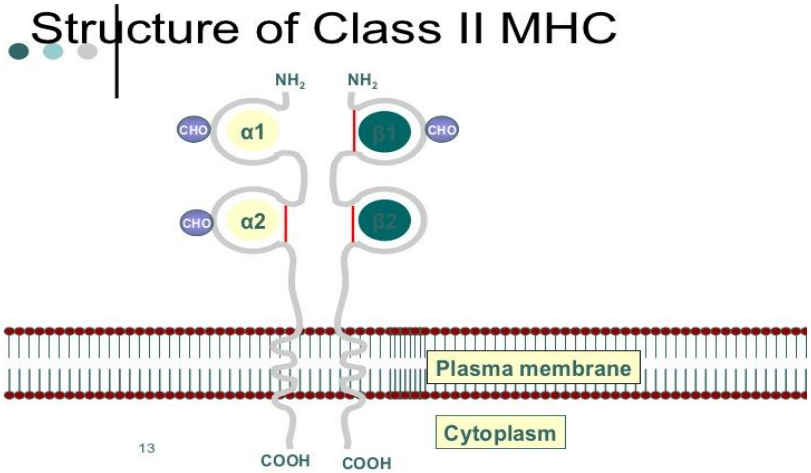
Essentially, all nucleated cells carry classical class I molecules. These are abundantly expressed on lymphoid cells, less so on liver, lung and kidney, and only sparsely on brain and skeletal muscle. In the human, the surface of the villous trophoblast lacks HLA-A and -B and bears HLA G, which does not appear on any other body cell. Class II molecules are also restricted in their expression, being present only on antigen presenting cells (APCs) such as B-cells, dendritic cells and macrophages and on thymic epithelium. When activated by agents such as interferon g, capillary endothelia and many epithelial cells in tissues other than the thymus, they can develop surface class II and increased expression of class I.

They function as cell surface markers enabling infected cells to signal cytotoxic and helper T-cells.

Importance of MHC

1. Antibody molecules interact with antigen directly but the T-Cell Receptor (TCR) only recognizes antigen presented by MHC molecules on another cell, the Antigen Presenting Cell. The TCR is specific for antigen, but the antigen must be presented on a self-MHC molecule.
2. The TCR is also specific for the MHC molecule. If the antigen is presented by another allelic form of the MHC molecule in vitro (usually in an experimental situation), there is no recognition by the TCR. This phenomenon is known as **MHC restriction**.



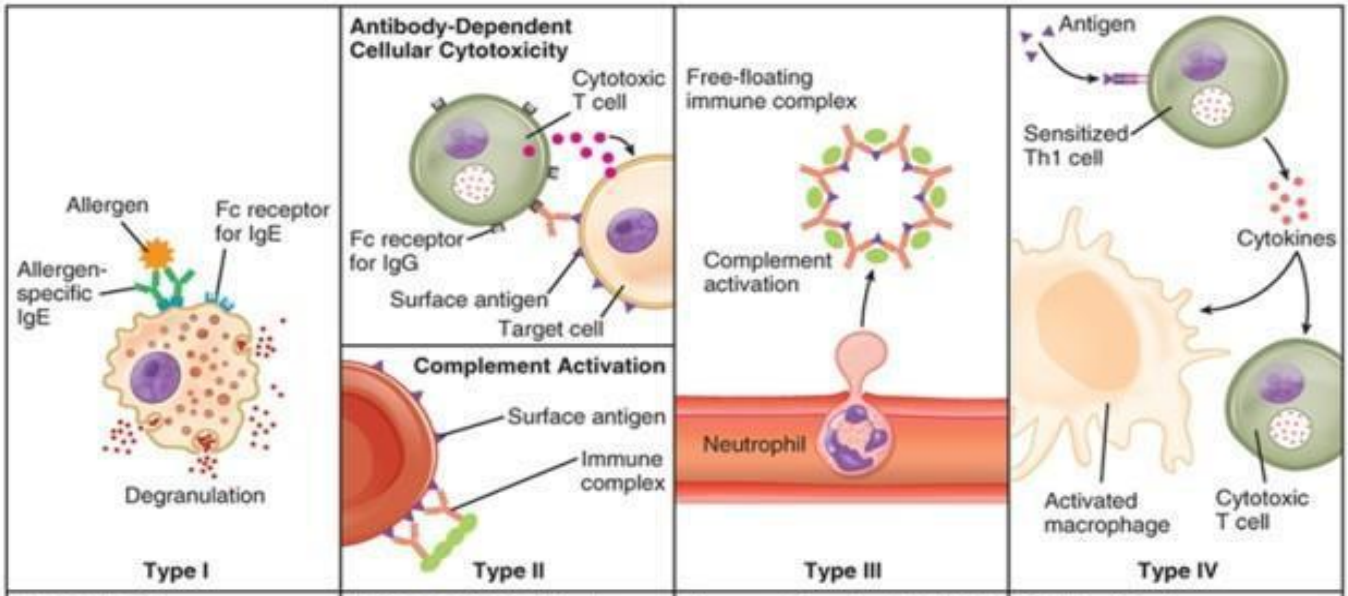


HYPERSENSITIVITY

The adaptive immunity, having a diversity of responses involved for its function, may be involved in quite a lot of problems when it comes to tissue injury and diseases. As much as it can protect the body from harmful pathogens, it can also be implicated in situations where there is an exaggerated response leading to tissue damage. These conditions are collectively called as **hypersensitivity reactions**.

Hypersensitivity reactions can be caused by a variety of reasons. For one, our **own immune system could react with the functioning cells** within our body. Under normal circumstances, the immune system employs a screening process wherein self-detecting immune cells are eliminated from the circulation. However, in some people, this mechanism can be absent.

Hypersensitivity can also be **activated in the actual presence of a microbe**. In this situation, there is an exaggeration in the efforts to neutralize the microorganism. This can lead to the destruction of the cells, tissues, and structures adjacent to the area of invasion. This mechanism is most pronounced when the microorganism is very persistent.



IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
IgE is bound to mast cells via its Fc portion. When an allergen binds to these antibodies, crosslinking of IgE induces degranulation.	Cells are destroyed by bound antibody, either by activation of complement or by a cytotoxic T cell with an Fc receptor for the antibody (ADCC)	Antigen–antibody complexes are deposited in tissues, causing activation of complement, which attracts neutrophils to the site	Th1 cells secrete cytokines, which activate macrophages and cytotoxic T cells and can cause macrophage accumulation at the site
Causes localized and systemic anaphylaxis, seasonal allergies including hay fever, food allergies such as those to shellfish and peanuts, hives, and eczema	Red blood cells destroyed by complement and antibody during a transfusion of mismatched blood type or during erythroblastosis fetalis	Most common forms of immune complex disease are seen in glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus	Most common forms are contact dermatitis, tuberculin reaction, autoimmune diseases such as diabetes mellitus type I, multiple sclerosis, and rheumatoid arthritis

Environmental antigens may also play a part in tissue destruction caused by the immune reaction. In select people, a certain antibody (**immunoglobulin E or IgE**) is produced in the presence of an allergen coming from the environment, leading to the development of an allergic reaction.

Type I

In Type 1 hypersensitivity reactions mast-cell activation is induced by secretion of IgE antibodies. Initial exposure to the antigen causes the priming of Th2 cells, and their release of IL-4 causes the B cells to switch their production of IgM to IgE antibodies which are antigen-specific. The IgE antibodies bind to **mast cells** and basophils, sensitising them to the antigen.

When the antigen enters the body again, it cross links the **IgE** bound to the sensitised cells, causing the release of preformed mediators including histamine, leukotrienes and prostaglandins. This leads to widespread vasodilation, bronchoconstriction, and increased permeability of vascular endothelium.

The reaction can be divided into two stages – immediate, in which release of pre-formed mediators causes the immune response, and the **late-phase response** 8-12 hours later, where cytokines released in the immediate stage activate basophils, eosinophils, and neutrophils even though the antigen is no longer present.

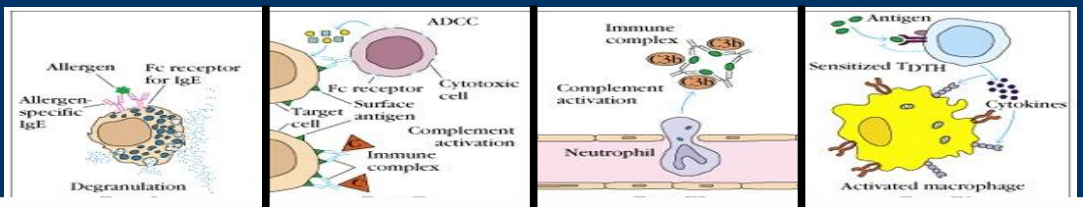
Some common examples of the **early-phase** of immediate hypersensitivity are:

- Rashes or blisters in the skin
- Presence of discharges from the eyes and nose
- Hay fever
- Bronchial asthma
- Gastrointestinal abnormalities (e.g. diarrhea, vomiting)

When activated circulating effector cells, such as mast cells, come into contact with the same allergen. This can cause them to go haywire by releasing a lot of mediators that can cause inflammation and tissue damage. These include **preformed mediators** (vasoactive amines, enzymes, and proteoglycans)**, lipid-derived mediators** (leukotrienes, prostaglandin D2 and platelet-activating factor) and other cytokines.

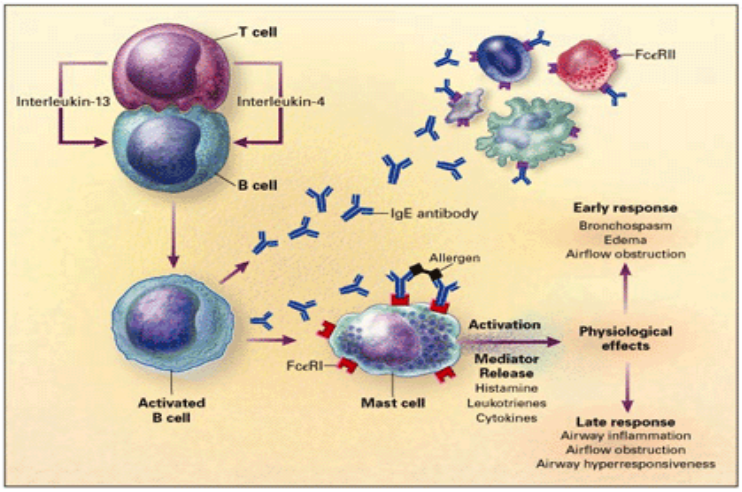
During the **late-phase** of an immediate hypersensitivity response, the reactions that led to the activation of effector cells and the production of potent mediators are sustained. This is made possible as additional cells of the immune system are recruited despite the already eliminated allergen. Eosinophils are usually the ones present in these cases.

Gel and Coombs classification of hypersensitivities.



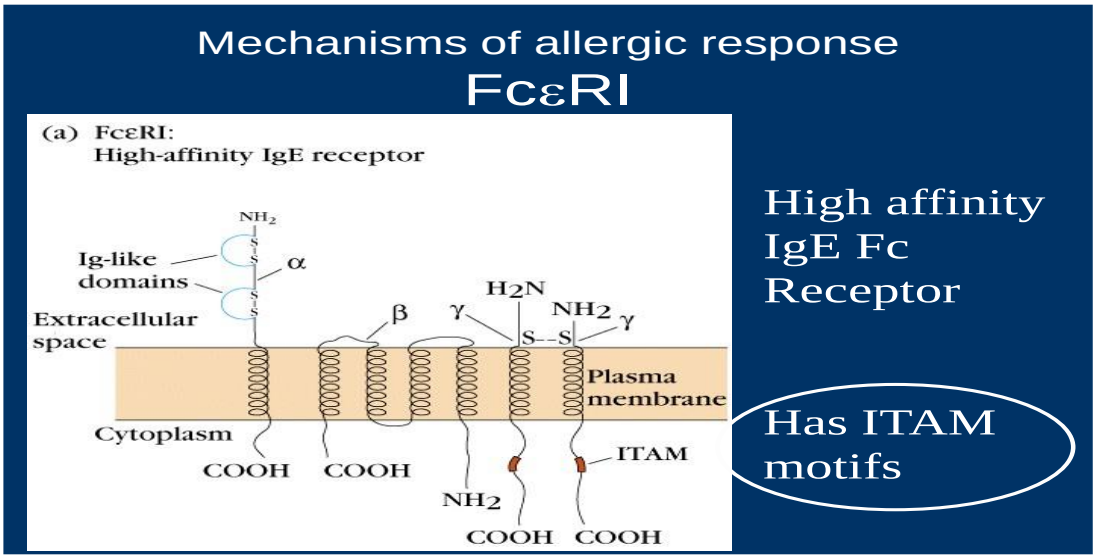
Type I	Type II	Type III	Type IV
IgE Mediated	IgG/IgM Mediated	IgG Mediated	T cell
Classic Allergy	rbc lysis	Immune complex Disease	Delayed Type Hypersensitivity

Mechanisms of allergic response
Sensitization
Th2/B cell interaction



IL-4
IL-4R
CD40
Drive B cell Activation and IgE isotype switch.

Busse and Lemanske NEJM Feb 2001. 344:350



Type II – antibody-dependent hypersensitivity

Type 2 hypersensitivity reactions are mediated by antibodies targeting antigens on cell surfaces. When cell surface antigens are presented to T cells, an immune response is started, targeting the cells to which the antigens are attached.

Antibodies binding to cells can activate the complement system, leading to degranulation of **neutrophils,** a release of oxygen radicals, and eventual formation of membrane attack complex – all of which lead to destruction of the cell. Parts of the complement activation can also opsonise the target cell, marking it for phagocytosis.

The destruction of host cells in this way can lead to tissue-specific damage. Type 2 hypersensitivity reactions may occur in response to host cells (i.e. autoimmune) or to non-self cells, as occurs in blood transfusion reactions.

Type 2 is distinguished from Type 3 by the location of the antigens – in Type 2, the antigens are cell bound, whereas in Type 3 the antigens are soluble.

Type III – immune complex-mediated hypersensitivity

Type 3 hypersensitivity reactions are mediated by antigen-antibody complexes (formed by soluble antigens) in the circulation that may be desposited in and damage tissues. The complexes may become lodged in the basement membranes of tissues which have particularly high rates of blood filtration – the kidney and synovial joints being common targets.

Once lodged, the immune complexes rapidly and significantly activate the **complement** chain, causing local inflammation and attraction of leucocytes. Activation of

complement results in increased vasopermeability, the attraction and degranulation of neutrophils, and the release of oxygen free radicals which can severely damage surrounding cells.

Acute serum sickness

Immune complex-mediated hypersensitivity reaction is very evident in cases where sera coming from different organisms or species (e.g. anti-tetanus immunoglobulin from horse serum) is injected into human beings (e.g. when a child punctured by a nail). This is commonly called **acute serum sickness.** Although very rare, this case serves as a model in explaining what goes on during a type III reaction.

Arthus reaction

Another case that exemplifies this type of reaction is the **Arthus phenomenon**. This occurs when a locally injected antigen causes a localized reaction that is seen as necrosis of the overlying tissues. This is due to the deposition of complexes on the vascular structures found in that area.

Type IV – T cell-mediated hypersensitivity

As the name implies, type IV hypersensitivity reactions are characterized by a rather delayed response mediated by either helper or cytotoxic T cells. In most cases, it is usually the helper T cells that are implicated in most cases of hypersensitivity. Type 4 hypersensitivity reactions are mediated by antigen-specific activated **T-cells.** When the antigen enters the body, it is processed by antigen-presenting cells and presented together with the MHC II to a Th1 cell.

If the T-helper cell has already been primed to that specific antigen, it will become activated and release chemokines to recruit macrophages and cytokines such as interferon-γ to activate them. Activated **macrophages** release pro-inflammatory factors, leading to local swelling, oedema, warmth, and redness. They also secrete lysosomal elements and reactive oxygen species, again leading to local tissue damage. CD8+ T cells may be involved in type 4 reactions where a foreign antigen is detected on a cell, such as in organ rejection: this is known as cell mediated cytotoxicity, and also results in recruitment and activation of macrophages.

The reaction takes longer than all other types because of the length of time required to recruit cells to the site of exposure – around 24 to 72 hours.

Cytotoxic T lymphocytes

Unlike helper T cells, cytotoxic T cells directly kill the cells that carry the triggering antigen. For this reason, this particular subtype is highly effective against viral and parasitic infections in which the offending agent is found intracellularly.

These cells do damage by releasing molecules and complexes that damage the microbe. Unfortunately, these substances are not selective only to offending agents and can be detrimental to surrounding healthy tissues. Cases where there is a cytotoxic T lymphocyte-mediated hypersensitivity are the following:

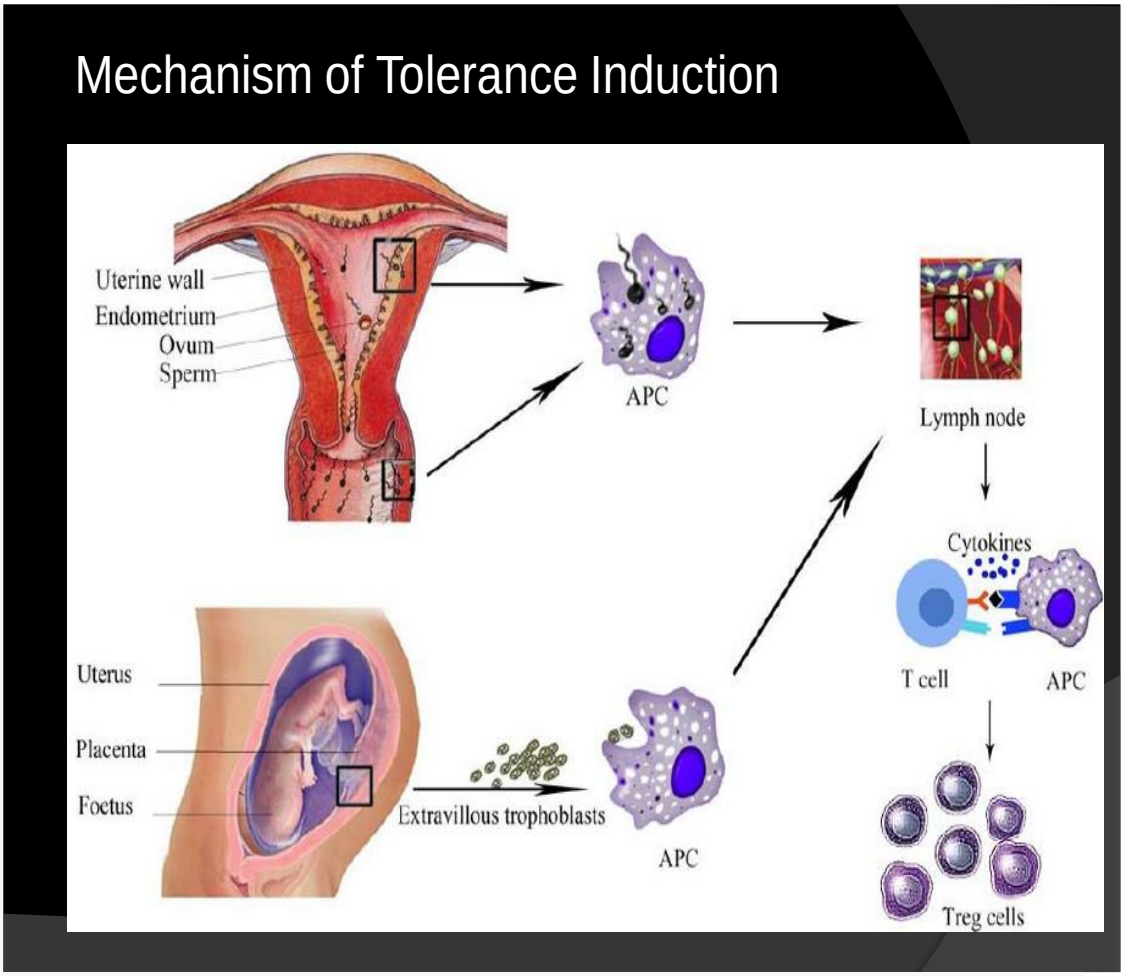
- Liver damage during viral hepatitis
- Organ graft rejection

IMMUNE TOLERANCE

Introduction

- Erlich put forward the term ‘horror autotoxicus’ to refer to the body’s aversion to immunological self destruction, and postulated that mechanisms must prevent reaction against self
- Healthy individuals have a smouldering autoimmune response that can escape control (a system of many checks and balances) to cause autoimmune disorders
- A major area of interest is how the immune system discriminates between self and non-self, and thus how to minimise the possibility of autoimmunity while optimising protective immune responses
- Active mechanisms also prevent an immunologic attack on harmless antigens such as those represented by commensal bacteria and food
- Control mechanisms induce a state of immune unresponsiveness to an antigen
- Tolerance is induced and maintained both centrally and peripherally, each with a non-redundant function in maintaining receptor diversity while curtailing self-reactivity

- It was observed more than 50 years ago that non-identical twin calves sharing a placenta do not react to each other's erythrocytes, and it was hypothesised that *in utero* exposure rendered them tolerant to each other's antigens
- This spurred Medawar and Brent to inject mouse cells into a neonatal mouse from a different strain, resulting in tolerance of the latter to skin grafts from the former ***[see their original paper, "Actively Acquired Tolerance of Foreign Cells"; downloadable at end of this section]***
- MacFarlane Burnet pioneered the clonal selection theory: where host encounter with a foreign antigen selects for a particular immune cell clone that then proliferates to yield daughter clones which all have the same specificity
- He hypothesised that self-tolerance resulted from central deletion of 'forbidden clones', thereby eliminating potentially self-reactive clones
- additionally, a deficiency in Treg number or function leads to allergic, autoimmune and other inflammatory diseases due to loss of tolerance
- The most important form of tolerance is self tolerance, which occurs in the developing foetus during normal immune ontogeny
- Tolerance can also be induced to non-self antigens after birth
- An antigen that induces tolerance is termed a tolerogen and tolerance is both antigen- and cell clone-specific: tolerance is selective for the tolerogen that induced it, facilitating continued responsiveness to other antigens
- In this way, tolerance is different from generalised immune suppression (such as that induced by post-transplant drugs like cyclosporine)

- 

Central vs. Peripheral Tolerance

- Induction of tolerance requires education of both B and T cells, which occurs in both central (bone marrow, thymus) and peripheral (spleen, lymph nodes) lymphoid organs and tissues
- Here lymphocytes become either immune competent or tolerant towards encountered antigens
- Mechanisms of tolerance induction and maintenance differ between B and T cells, and between central and peripheral lymphoid organs

Central T-Cell Selection

- Transgenic animal models demonstrate that central mechanisms are indispensable for induction of self-tolerance

- CD4-CD8- (double negative) T-cell progenitors enter the thymic cortex and rearrange their receptors to become CD4+CD8+ (double positive) thymocytes
- Positive and negative selection occurs in the thymus.
- T-cells with a receptor that bind with moderate affinity to self-peptide-MHC complexes on thymic epithelia receive a survival signal (positive selection)
- Depending on which MHC was recognised, the T-cell will display either CD4 or CD8 (single positive)
- Negative selection occurs at the DP stage in the cortex, or at the SP stage in the medulla: T-cells with a receptor that bind with high avidity to autoantigens on thymic epithelia undergo apoptosis
- The autoantigens are host tissue proteins expressed on thymic epithelia under regulation of the transcription factor autoimmune regulator (AIRE)
- Many T-cells are eliminated: of the potential 109 receptor specificities in the thymus, only a fraction are present in peripheral tissues
- AIRE deficiency results in organ-specific autoimmunity, including **APS-1** (damage to parathyroid and adrenal glands)

Peripheral T-Cell Selection

- Central and Peripheral tolerance occur in tandem, in the case that central tolerance is not completely effective; partly because not all autoantigens are expressed in the thymus
- Several autoreactive clones are found in the peripheral blood of healthy people, and some lymphocytes from people without MS react in vitro to MBP (a target of the immune response in MS)
- Autoreactive clones can potentially become activated and proliferate in the periphery when properly stimulated (e.g. sub-acute bacterial endocarditis can lead to emergence of self-reactive clones that damage the kidneys)
- Peripheral mechanisms of tolerance eliminate or suppress autoreactive clones that escape to the periphery

- Mechanisms of peripehral T-cell tolerance include:
    - A. Clonal deletion
    - B. Ignorance
    - C. Anergy
    - D. Immune regulation
- Tolerance mechanisms can also result in inappropriate tolerance to non-self antigens.
- As an example: when LCM virus was inoculated into mouse embryos, adult mice mounted no immune response to LCM and were chronically infected
- It was thought that these antigens, introduced early during gestation, were handled as self-antigens, thereby inducing negative central T-cell selection.

A. Clonal Deletion

- The best-studied mechanism eliminating activated T-cell clones is activation-
- of death receptors (e.g. Fas) and their ligands
- Ligation of Fas leads to T-cell apoptosis via the caspase pathway, thereby ending the immune response

Mutations in Clonal Deletion Pathways

- Mice whose T-cells do not express Fas, display expanded lymphocyte populations in their secondary lymphoid organs, leading to profound lymphadenopathy, significant self-reactivity (including many autoantibodies), and autoimmune damage to many organs (including kidneys)
- Rare human diseases, **ALPS** 1a and 1b, are caused by similar mutations in Fas or FasL respectively, also leading to lymphadenopathy and autoantibodies
- IL-2 affects the Fas pathway, and can eventually lead to AICD by increasing FasL expression

- IL-2 also helps attenuate immunity by downregulating survival molecules (e.g. FLICE and FLIP) which would otherwise inhibit AICD by preventing assembly of the Fas death receptor complex

B. Ignorance

- Although peripherally, T-cells from healthy individuals can react with self-antigens in vitro, this does not commonly occur in vivo
- It is thought that T-cells ignore certain self-antigens because they are located in immune-privileged sites or because they have low immunogencitiy (low levels of expression or low binding affinity)
- **Sympathetic autoimmune ophthalmia** (Case 38 in *Bellanti JA (Ed). Immunology IV: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, 2012 and* related **Case Study – Case of eye injury and decreased vision**) – severe inflammatory damage to both eyes – is caused by release of sequestered ocular self-antigens into circulation, where they can eventually activate peripheral autoreactive immune cells
- the immune system is not normally exposed to ocular antigens, but trauma to a single eye releases autoantigens that activate autoreactive immune cells, leading to severe granulomatous inflammatory of both eyes

C. Anergy

- This is a major mechanism inactivating peripheral autoreactive T-cell clones
- Anergic T-cell clones cannot respond to cognate antigenic stimuli: they do not produce IL-2 or IL-2R
- Multiple proposed mechanisms explain this block in T-cell activation (FIGURE **3**):
- Disruption of the interaction between the T-cell co-receptor CD28 and APC co-stimualtory molecules CD80/86
- Interaction of CTLA-4 with CD80/86, negatively regulating T-cell activation

- T-cells displaying CD28 tend to be activated by APC, while T-cells displaying CTLA-4 tend to become anergic
- Under physiologic conditions, T-cells express CD28 on initial encounter with APC
- Shortly after such stimulation, they start displaying CTLA-4, which has a higher binding affinity than CD28 for CD80/86 than does CD28
- CTLA-4 knockout mice develop profound lympho-proliferative disease

Clinical Significance of CTLA-4

- Rheumatoid arthritis is an autoimmune disease mainly affecting the joints
- CTLA-4 is used as a biologic response modifier to treat these patients, significantly reducing joint inflammation
- A fusion protein consisting of CTLA-4 and immunoglobulin (abatacept, belatacept) is used clinically in arthritis and after transplantation
- There is also interest in blocking CTLA-4 using monoclonal antibodies (e.g. ipilimumab) to inhibit tumor tolerance
- Insufficient production of key transcriptional activators (e.g. AP-1, NF-kB, NFAT-1) during T-cell activation reduces IL-2 production
- Transcription suppressors (e.g. CREM) can also decrease transcriptional activity of the IL-2 gene promoter, leading to T-cell anergy
- Alterations at multiple levels of regulation (e.g. activity of key kinases such as MAPK, or stability of IL-2 mRNA) can contribute to reduced IL-2 production in anergic T-cells

D. Immune Regulation

- Immune regulation is achieved by the action of Treg's
- Treg are important in the maintenance of peripheral tolerance
- When they are depleted from mice, autoimmunity results

- A human patient with genetic Treg dysfunction develops lymphadenopathy and inflammatory infiltrates consisting of autoreactive T-cells in multiple organs
- Naturally-occurring thymus-derived Treg display anergic properties *in vitro*, but can also suppress CD4+CD25- T-cells *in vivo*, via direct cell-cell contact, or secretion of cytokines (FIGURE 4)
- During active inflammation (e.g. infection), Treg do not prevent protective immune function
- Induced CD4+CD25+ Treg can also be activated in peripheral lymphoid organs (e.g. by TGFβ) and suppress immune responses via anti-inflammatory cytokines (e.g. TGFβ) rather than direct contact.

TGFβ & Regulatory Cells Other Than Treg

- TGFβ in the presence of IL-6 (e.g. from activated macrophages during infection) and IL-23 can also lead to induction of Th17, which produce IL-17
- Th17 are associated with antimicrobial immunity as well as autoimmune/inflammatory disorders
- TGFβ can therefore either regulate inflammation through Treg, or promote inflammation through Th17
- CD1-restricted NKT cells have also been implicated in immune regulation, as have CD8+ suppressor T-cells
- γδCD8+ T-cells that populate MALT are most likely involved in suppression of immune responses initiated by antigen delivered by the mucosal route
- After inhalation of small quantities of antigen, such CD8+ T-cells are activated in sub-mucosal areas to become suppressors cells and migrate to draining lymph nodes to suppress immune response via production of IL-10 and TGFβ
- Ingestion of larger quantities of antigen activates not only such CD8+ suppressors, but also Treg that migrate to areas of inflammation to downregulate T-driven immune responses

- Th1-type IFNγ opposes Th2-type immunity, while Th2-type IL-4 opposes Th1-type immunity
- Regulatory T-cells and cytokines are also being used as and targeted therapeutically

B-cell Tolerance

During normal B-cell development, a set of processes help induce B-cell central tolerance

- Education of B-cells and elimination of self-reactive B-cell clones is somewhat different from that of T-cells
- B-cells are still immature when they relocate from bone marrow to spleen T-cell zones
- Autoreactive B-cells are not necessarily eliminated during negative selection in the bone marrow
- B-cells that recognise autoantigens are eliminate via apoptosis or become anergic
- Autoreactive B-cells that escape negative selection become part of the a maximally-diverse immune repertoire

B-cell Peripheral Tolerance

- Peripheral tolerance mechanisms (in secondary lymphoid tissues) exist for various reasons:
- Imperfect T-cell tolerance: in most autoimmune diseases, B-cells are T-cell dependent, requiring help from pre-activated cognate autoreactive T-cells
- T-independent B-cells can be activated by autoantigens without T-cell help
- Microbial antigens structurally similar to autoantigens can lead B-cells to produce cross-reactive antibodies in a phenomenon known as molecular mimicry
- B-cells hypermutate their receptors on activation, so there is a second chance that they may become self-reactive

Exploring B-cell Tolerance

- Mechanisms of B-cell tolerance have been explored using transgenic mice (FIGURE **5**)
- In a classic experiment, monotransgenic mice expressing hen egg lysozyme (after tolerisation via fetal exposure) are bred with monotransgenic mice expressing anti-lysozyme IgM and having B-cell receptors capable of recognising lysozyme
- The resulting mouse hybrids produce lysozyme, but despite lysozyme-recognising B-cells do not produce anti-lysozyme antibody after immunization with lysozyme
- This demonstrates that the simultaneous presence of lysozyme and lysozyme-reactive B-cells leads to B-cell anergy
- B-cells from the hybrid strain transferred into irradiated wild-type mice were indeed able to make anti-lysozyme antibodies
- This proves that anergy was related to continued stimulation by lysozyme, but could be reversed when B-cells were in a host without the tolerising effects of lysozyme

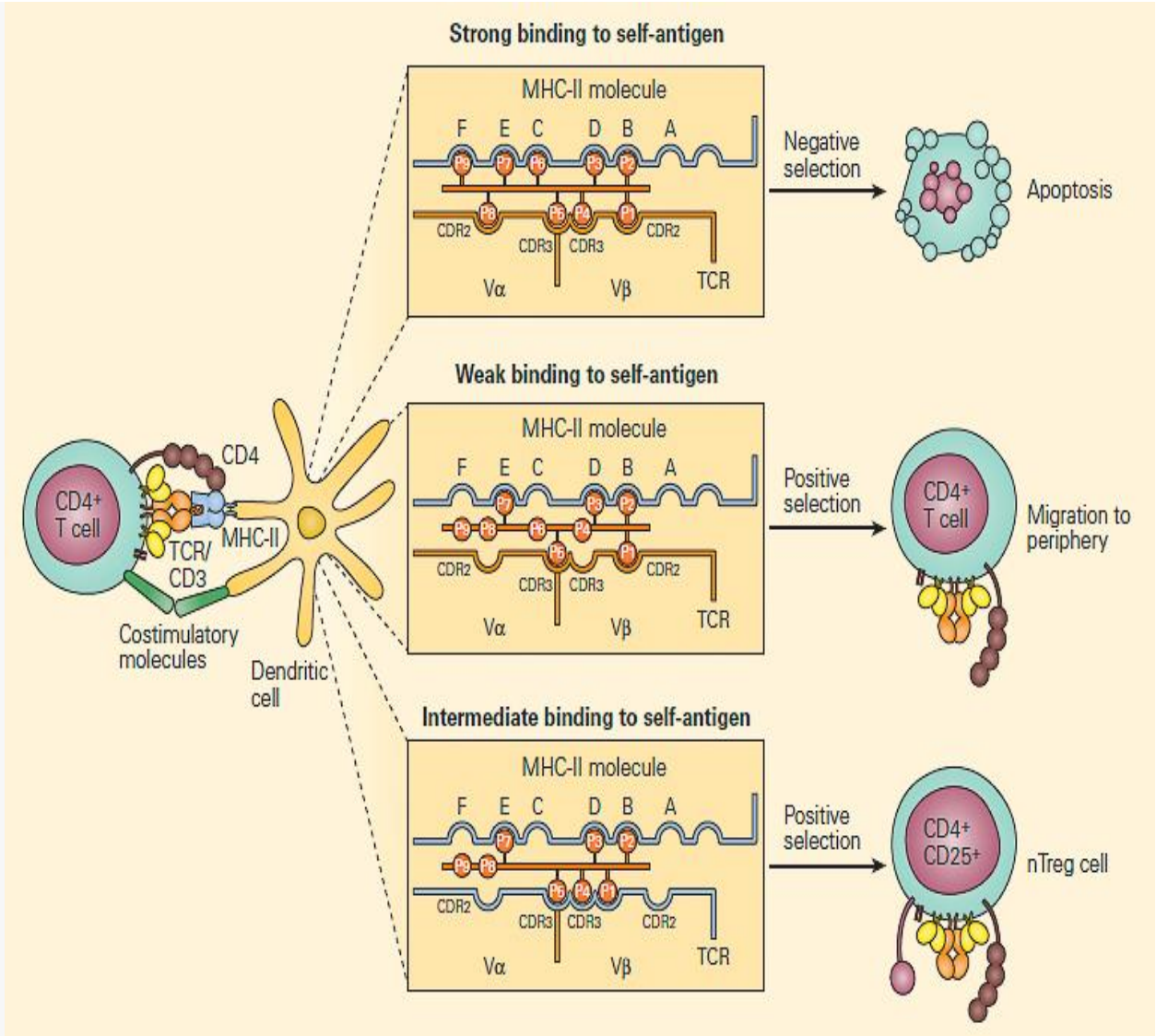
Regulatory B Cells & Tolerance

- B cells are known to be antibody producers. In fact more specifically, B stimulation occurs in the Germinal Centres of the lymph nodes and develop into antibody secreting plasma cells with the help of T follicular cells.
- This is a well described pathway and there are many reviews on the topic
- What is less well known, is the regulatory nature of B cells and how these cells can play a role in regulating immunity, additional to the antibody functions of these cells.

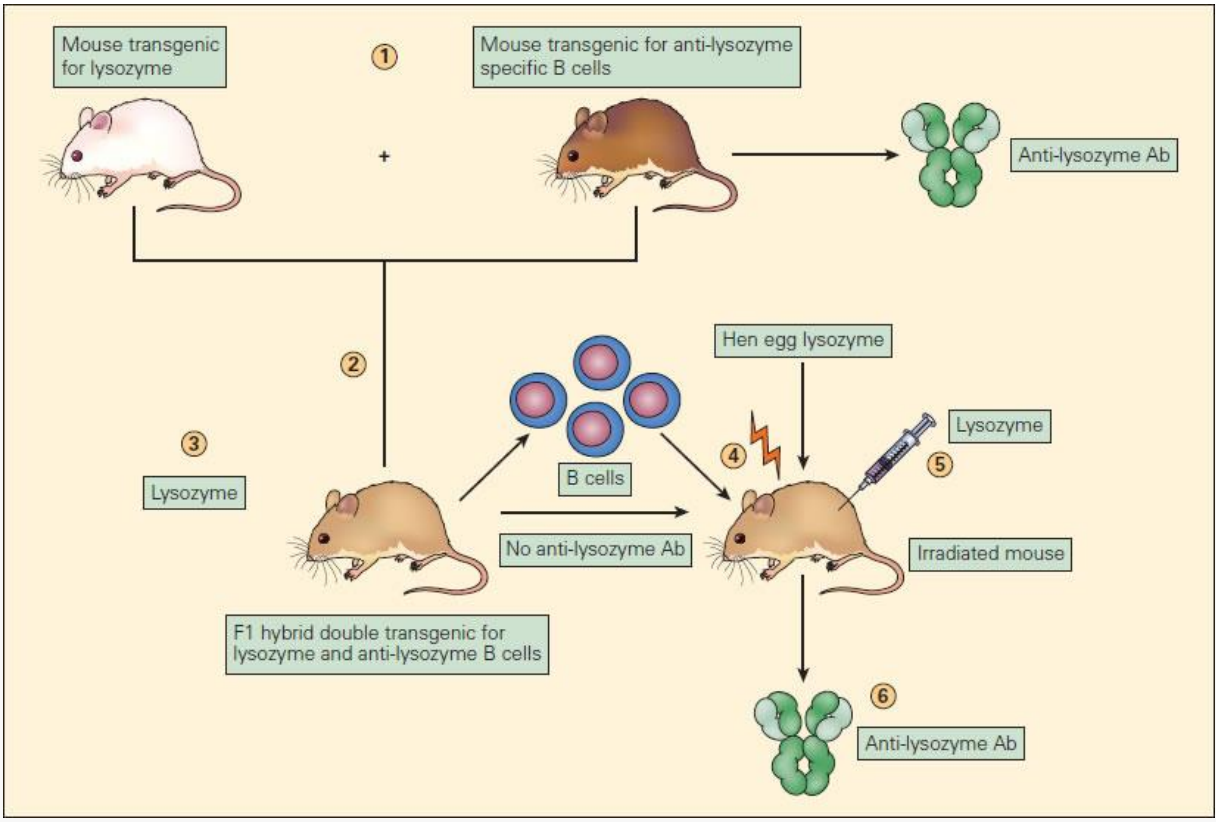
Autoimmunity: a breakdown of tolerance

- The immune system's three basic functions are Defence, Surveillance and Homeostasis
- Elaborate and redundant tolerance mechanisms are in effect both during maturation and later in the lymphocyte life-cycle, leading to clonal deletion or anergy

- Anergy can be reversed to allow recruitment of autoreactive clones to maximise receptor diversity (e.g. during infection)
- Autoimmunity [Table 1] emerges when self-tolerance mechanisms fail (infections can lead to breaking of tolerance [**Case Studies – A 9 year old girl presents with body swelling, shortness of breath and backache** and **Why can I not walk today?**]
- Primary immunodeficiencies can also present with altered surveillance (leading to malignancy) or altered homeostasis (leading to autoimmunity)
- Breaking tolerance usually occurs as a consecutive series of many events (rarely due to a single genetic/environmental factor).
- Breaking of tolerance can be conceptualised as being set in motion by just the right stimuli occurring against the backdrop of a predisposing immunological milieu
- This milieu is influenced by genetics, prior antigen encounter, local factors in target organs, and other endogenous factors (e.g. the immune-modulating effects of hormones such as cortisol and oestrogen)
- Autoreactive T-cells can, for example, proliferate peripherally during an infection or on release of previously-sequestered antigens, or defects of apoptosis can facilitate development of T-cell autoreactivity
- Similar mechanisms can lead to proliferation of autoreactive B-cells



Positive and negative selection in the thymus. CD4+ T cells that recognize self-antigens expressed on thymocytes in the context of MHC-II molecules undergo apoptosis. The key factor in determining positive and negative selection is the strength of the antigen recognition by the maturing T cell; low-avidity recognition leads to positive selection, and high-avidity recognition induces negative selection. It is proposed that at this stage, Treg (CD4+CD25+) cells that are autoantigen-specific are generated by intermediate degrees of binding. [Reproduced with permission from Bellanti JA (Ed). Immunology IV: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, 2012]



Experimental model of B cell anergy. (1) A transgenic mouse expressing hen egg lysozyme (white mouse) was crossed with a mouse transgenic for anti-lysozyme specific B cell production (brown mouse) to produce an F1 hybrid (tan mouse) (2) double transgenic for lysozyme production (3) and anti-lysozymal B cell production; these double transgenic hybrids produced lysozyme but did not produce anti-lysozyme antibody. (4) The B cells from these F1 mice, although unable to react to the lysozyme, when transferred to irradiated mice and immunized with lysozyme (5) resulted in the production of anti-lysozyme antibody (Ab) (6). This proves that the B cells in the F1 mice, although capable of producing anti-lysozyme antibody, if transferred to another mouse were functionally anergized in the presence of endogenously produced lysozyme [Reproduced with permission from Bellanti JA (Ed). Immunology IV: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, 2012].

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