Vol 2|Issue 2| 2012 | 62-70.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

AN OVERVIEW OF IMMUNOLOGY

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ABSTRACT

Immunology is a branch of biomedical science that covers the study of all aspects of the immune system in all organisms. This review deals with the physiological functioning of the immune system in states of both health and diseases; malfunctions of the immune system in immunological disorders (autoimmune diseases, hypersensitivities, immune deficiency, transplant rejection); the physical, chemical and physiological characteristics of the components of the immune system in vitro, in situ, and in vivo. Immunology has applications in several disciplines of science.

Keywords: Immunology, Application, Types, ELISA.

INTRODUCTION

Even before the concept of immunity was developed, numerous early physicians characterized organs that would later prove to be part of the immune system. The key primary lymphoid organs of the immune system are the thymus and bone marrow, and secondary lymphatic tissues such as spleen, tonsils, lymph vessels, lymph nodes, adenoids, and skin and liver. When health conditions warrant, immune system organs including the thymus, spleen, portions of bone marrow, lymph nodes and secondary lymphatic tissues can be surgically excised for examination while patients are still alive. Many components of the immune system are actually cellular in nature and not associated with any specific organ but rather are embedded or circulating in various tissues located throughout the body.

Classical immunology

Classical immunology ties in with the fields of epidemiology and medicine. It studies the relationship between the body systems, pathogens, and immunity. The earliest written mention of immunity can be traced back to the plague of Athens in 430 BCE. Thucydides noted that people who had recovered from a previous bout of the disease could nurse the sick without contracting the illness a second time. Many other ancient societies have references to this phenomenon, but it was not until the 19th and 20th centuries before the concept developed into scientific theory.

The study of the molecular and cellular components that comprise the immune system, including their function and interaction, is the central science of immunology. The immune system has been divided into a more primitive innate immune system, and acquired or adaptive immune system of vertebrates, the latter of which is further divided into humoral and cellular components [1].

The humoral (antibody) response is defined as the interaction between antibodies and antigens. Antibodies are specific proteins released from a certain class of immune cells (B lymphocytes). Antigens are defined as anything that elicits generation of antibodies, hence they are Antibody Generators. Immunology itself rests on an understanding of the properties of these two biological entities. However, equally important is the cellular response, which can not only kill infected cells in its own right, but is also crucial in controlling the antibody response. Put simply, both systems are highly interdependent.

In the 21st century, immunology has broadened its horizons with much research being performed in the more specialized niches of immunology. This includes the immunological function of cells, organs and systems not

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normally associated with the immune system, as well as the function of the immune system outside classical models of immunity.

Clinical immunology

Clinical immunology is the study of diseases caused by disorders of the immune system (failure, aberrant action, and malignant growth of the cellular elements of the system). It also involves diseases of other systems, where immune reactions play a part in the pathology and clinical features.

The diseases caused by disorders of the immune system fall into two broad categories: immunodeficiency, in which parts of the immune system fail to provide an adequate response (examples include chronic granulomatous disease), and autoimmunity, in which the immune system attacks its own host's body (examples include systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's disease and myasthenia gravis). Other immune system disorders include different hypersensitivities, in which the system responds inappropriately to harmless compounds (asthma and other allergies) or responds too intensely.

The most well-known disease that affects the immune system itself is AIDS, caused by HIV. AIDS is an immunodeficiency characterized by the lack of CD4+ (helper) T cells, dendritic cells and macrophages, which are destroyed by HIV. Clinical immunologists also study ways to prevent transplant rejection, in which the immune system attempts to destroy allografts.

Developmental immunology

The body's capability to react to antigen depends on a person's age, antigen type, maternal factors and the area where the antigen is presented. Neonates are said to be in a state of physiological immunodeficiency, because both their innate and adaptive immunological responses are greatly suppressed. Once born, a child's immune system responds favorably to protein antigens while not as well to glycoproteins and polysaccharides. In fact, many of the infections acquired by neonates are caused by low virulence organisms like Staphylococcus and Pseudomonas. In neonates, opsonic activity and the ability to activate the complement cascade is very limited. For example, the mean level of C3 in a newborn is approximately 65% of that found in the adult. Phagocytic activity is also greatly impaired in newborns. This is due to lower opsonic activity, as well as diminished up-regulation of integrin and selectin receptors, which limit the ability of neutrophils to interact with adhesion molecules in the endothelium. Their monocytes are slow and have a reduced ATP production, which also limits the newborns phagocytic activity. Although, the number of total lymphocytes is significantly higher than in adults, the cellular and humoral immunity is also impaired. Antigen presenting cells in newborns have a reduced capability to activate T cells. Also, T cells of a newborn proliferate poorly and produce very small amounts of cytokines like IL-2, IL-4, IL-5, IL-12, and IFN-g which limits their capacity to activate the humoral response as well as the phagocitic activity of macrophage. B cells develop early in gestation but are not fully active [2].

Maternal factors also play a role in the body's immune response. At birth most of the immunoglobulin is present is maternal IgG. Because IgM, IgD, IgE and IgA don't cross the placenta, they are almost undetectable at birth. Although some IgA is provided in breast milk. These passively acquired antibodies can protect the newborn up to 18 months, but their response is usually short-lived and of low affinity. These antibodies can also produce a negative response. If a child is exposed to the antibody for a particular antigen before being exposed to the antigen itself then the child will produce a dampened response. Passively acquired maternal antibodies can suppress the antibody response to active immunization. Similarly the response of T-cells to vaccination differs in children compared to adults, and vaccines that induce Th1 responses in adults do not readily elicit these same responses in neonates. By 6-9 months after birth, a child's immune system begins to respond more strongly to glycoproteins. Not until 12-24 months of age is there a marked improvement in the body's response to polysaccharides. This can be the reason for the specific time frames found in vaccination schedules.

During adolescence the human body undergoes several physical, physiological and immunological changes. These changes are started and mediated by different hormones. Depending on the sex either testosterone or 17-β-oestradiol, act on male and female bodies accordingly, start acting at ages of 12 and 10 years. There is evidence that these steroids act directly not only on the primary and secondary sexual characteristics, but also have an effect on the development and regulation of the immune system. There is an increased risk in developing autoimmunity for pubescent and post pubescent females and males. There is also some evidence that cell surface receptors on B cells and macrophages may detect sex hormones in the system.
The female sex hormone 17-β-oestradiol has been shown to regulate the level of immunological response. Similarly, some male androgens, like testosterone, seem to suppress the stress response to infection; but other androgens like DHEA have the opposite effect, as it increases the immune response instead of down playing it. As in females, the male sex hormones seem to have more control of the immune system during puberty and the time right after than in fully developed adults. Other than hormonal changes physical changes like the involution of the Thymus during puberty will also affect the immunological response of the subject or patient.

Immunotherapy

The use of immune system components to treat a disease or disorder is known as immunotherapy. Immunotherapy is most commonly used in the context of

the treatment of cancers together with chemotherapy (drugs) and radiotherapy (radiation). However, immunotherapy is also often used in the immunosuppressed (such as HIV patients) and people suffering from other immune deficiencies or autoimmune diseases [3].

Table 1. Immunomodulators

Agent	Example
Interleukins	IL-2, IL-7, IL-12
Cytokines	Interferons, G-CSF, Imiquimod
Chemokines	CCL3, CCL26, CXCL7
Other	Cytosine phosphate-guanosine, oligodeoxynucleotides, glucans

The active agents of immunotherapy are collectively called immunomodulators. They are a diverse array of recombinant, synthetic and natural preparations, often cytokines. Some of these substances, such as granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod and cellular membrane fractions from bacteria are already licensed for use in patients. Others including IL-2, IL-7, IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG), oligodeoxynucleotides and glucans are currently being investigated extensively in clinical and preclinical studies. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases.

Cell based Immunotherapies are proven to be effective for some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NK Cell), cytotoxic T lymphocytes (CTL), etc., work together to defend the body against cancer by targeting abnormal antigens expressed on the surface of the tumor due to mutation.

Activation immunotherapies

Cancer

Cancer immunotherapy attempts to stimulate the immune system to reject and destroy tumors. Dr William Coley used Coley's Toxins in the late 1800s as crude immunotherapy with some success. Immuno cell therapy for cancer was first introduced by Rosenberg and his colleagues of National Institute of Health USA. In the late 80s, they published an article in which they reported a low tumor regression rate (2.6–3.3%) in 1205 patients with metastatic cancer who underwent different types of active specific immunotherapy (ASI), and suggested that immuno cell therapy along with specific chemotherapy is the future of cancer immunotherapy. Initially Immunotherapy treatments involved administration of cytokines such as Interleukin. Thereafter the adverse effects of such intravenously administered cytokines lead to the extraction of the lymphocytes from the blood and expanding in vitro against tumour antigen before injecting the cells with appropriate stimulatory cytokines. The cells will then specifically target and destroy the tumor expressing antigen against which they have been raised.

The concept of this treatment started in the US in 80s and fully fledged clinical treatments on a routine basis have been in practice in Japan since 1990. Randomized controlled studies in different cancers resulting in significant increase in survival and disease free period have been reported and its efficacy is enhanced by 20–30% when cell-based immunotherapy is combined with other conventional treatment methods.

BCG immunotherapy for early stage (non-invasive) bladder cancer utilizes instillation of attenuated live bacteria into the bladder, and is effective in preventing recurrence in up to two thirds of cases. Topical immunotherapy utilizes an immune enhancement cream (imiquimod) which is an interferon producer causing the patients own killer T cells to destroy warts, actinic keratoses, basal cell cancer, vaginal intraepithelial neoplasia, squamous cell cancer, cutaneous lymphoma, and superficial malignant melanoma. Injection immunotherapy uses mumps, candida the HPV vaccine or trichophytin antigen injections to treat warts (HPV induced tumors). Lung cancer has been demonstrated to potentially respond to immunotherapy [4].

Dendritic cell-based immunotherapy

Dendritic cells can be stimulated to activate a cytotoxic response towards an antigen. Dendritic cells, a type of antigen presenting cell, are harvested from a patient. These cells are then either pulsed with an antigen or transfected with a viral vector. Upon transfusion back into the patient these activated cells present tumour antigen to effector lymphocytes (CD4+ T cells, CD8+ T cells, and B cells). This initiates a cytotoxic response to occur against cells expressing tumour antigens (against which the adaptive response has now been primed). The Dendreon cancer vaccine Provenge is one example of this approach.

T-cell adoptive transfer

Adoptive cell transfer uses T cell-based cytotoxic responses to attack cancer cells. T cells that have a natural or genetically engineered reactivity to a patient's cancer are generated in vitro and then transferred back into the cancer patient. One study using autologous tumor-infiltrating lymphocytes was an effective treatment for patients with metastatic melanoma. This can be achieved by taking T cells that are found with the tumor of the patient, which are trained to attack the cancerous cells. These T cells are referred to as tumor-infiltrating lymphocytes (TIL) are then encouraged to multiply in vitro using high concentrations of IL-2, anti-CD3 and allo-reactive feeder cells. These T cells are then transferred back into the patient along with exogenous administration of IL-2 to further boost their anti-cancer activity.

Thus far, a 51% objective response rate has been observed; and in some patients, tumors shrank to undetectable size. The initial studies of adoptive cell transfer using TIL, however, revealed that persistence of the transferred cells in vivo was too short. Before reinfusion, lymphodepletion of the recipient is required to eliminate regulatory T cells as well as normal endogenous lymphocytes that compete with the transferred cells for homeostatic cytokines. Lymphodepletion was made by total body irradiation prior to transfer of the expanded TIL. The trend for increasing survival as a function of increasing lymphodepletion was highly significant (P=0.007). Transferred cells expanded in vivo and persisted in the peripheral blood in many patients, sometimes achieving levels of 75% of all CD8+ T cells at 6–12 months after infusion.

Genetically engineered T cells

Genetically engineered T cells are created by infecting patient's cells with a virus that contain a copy of a T cell receptor (TCR) gene that is specialised to recognise tumour antigens. The virus is not able to reproduce within the cell however integrates into the human genome. This is beneficial as new TCR gene remains stable in the T-cell. A patient's own T cells are exposed to these viruses and then expanded non-specifically or stimulated using the genetically engineered TCR. The cells are then transferred back into the patient and ready to have an immune response against the tumour. Morgan et al. demonstrated that the adoptive cell transfer of lymphocytes transduced with retrovirus encoding TCRs that recognize a cancer antigen are able to mediate anti-tumour responses in patients with metastatic melanomas. This therapy has been demonstrated to result in objective clinical responses in patients with refractory stage IV cancer. The Surgery Branch of the National Cancer Institute (Bethesda, Maryland) is actively investigating this form of cancer treatment for patients suffering aggressive melanomas. The use of adoptive cell transfer with genetic engineered T cells is a promising new approach to the treatment of a variety of cancers.

In one case study, United States doctors from the Clinical Research Division, led by Dr. Cassian Yee at Fred Hutchinson Cancer Research Center in Seattle had successfully treated a patient with advanced skin cancer by injecting the patient with immune cells cloned from his own immune system. The patient was free from tumours within eight weeks of treatment. Dr. Cassian Yee described the research findings at The Cancer Research Institute International 2008 Symposia Series. Responses, however, were not seen in other patients in this clinical trial. Larger trials are now under way [5].

Immune recovery

The potential use of immunotherapy to restore the immune system of patients with immune deficiencies as result of infection or chemotherapy. For example cytokines have been tested in clinical trials interleukin-7 has been in clinical trials for HIV and cancer patients. In addition, interleukin-2 has also been tested in HIV patients.

Vaccination

Anti-microbial immunotherapy, which includes vaccination, involves activating the immune system to respond to an infectious agent.

Suppression immunotherapies

Immune suppression dampens an abnormal immune response in autoimmune diseases or reduces a normal immune response to prevent rejection of transplanted organs or cells.

Immune tolerance

Immune tolerance is the process by which the body naturally does not launch an immune system attack on its own tissues. Immune tolerance therapies seeks to reset the immune system so that the body stops mistakenly attacking its own organs or cells in autoimmune disease or accepts foreign tissue in organ transplantation. A brief treatment should then reduce or eliminate the need for lifelong immunosuppression and the chances of attendant side effects, in the case of transplantation, or preserve the body's own function, at least in part, in cases of type 1 diabetes or other autoimmune disorders.

Allergies

Immunotherapy is also used to treat allergies. While other allergy treatments (such as antihistamines or corticosteroids) treat only the symptoms of allergic disease, immunotherapy is the only available treatment that can modify the natural course of the allergic disease, by reducing sensitivity to allergens.

A one-to-five-year individually tailored regimen of injections may result in long-term benefits. Recent research suggests that patients who complete immunotherapy may continue to see benefits for years to come. Immunotherapy does not work for everyone and is only partly effective in some people, but it offers allergy sufferers the chance to eventually reduce or stop symptomatic/rescue medication.
The therapy is indicated for people who are extremely allergic or who cannot avoid specific allergens. For example, they may not be able to live a normal life and completely avoid pollen, dust mites, mold spores, pet dander, insect venom, and certain other common triggers of allergic reactions. Immunotherapy is generally not indicated for food or medicinal allergies. Immunotherapy is typically individually tailored and administered by an allergist (allergologist) or through a United Allergy Services lab available through specialized physician offices. Injection schedules are available in some healthcare systems and can be prescribed by family physicians. This therapy is particularly useful for people with allergic rhinitis or asthma [6].

The therapy is particularly likely to be successful if it begins early in life or soon after the allergy develops for the first time. Immunotherapy involves a series of injections (shots) given regularly for several years by a specialist in a hospital clinic. In the past, this was called a serum, but this is an incorrect name. Most allergists now call this mixture an allergy extract. The first shots contain very tiny amounts of the allergen or antigen to which you are allergic. With progressively increasing dosages over time, your body will adjust to the allergen and become less sensitive to it. This process is called desensitization. A recently approved sublingual tablet (Grazax), containing a grass pollen extract, is similarly effective, with few side effects, and can be self-administered at home, including by those patients who also suffer from allergic asthma, a condition which precludes the use of injection-based desensitization. To read more about this topic, see: allergy and hyposensitization.

Other approaches

Helminthic therapies

Recent research into the clinical effectiveness of Whipworm ova (Trichuris suis) and Hookworm (Necator americanus) for the treatment of certain immunological diseases and allergies means that these organisms must be classified as immuno-therapeutic agents. Helminthic therapy is being investigated as a potentially highly effective treatment for the symptoms and or disease process in disorders such as relapsing remitting multiple sclerosis Crohn's, allergies and asthma. The precise mechanism of how the helminths modulate the immune response, ensuring their survival in the host and incidentally effectively modulating autoimmune disease processes, is currently unknown. However, several broad mechanisms have been postulated, such as a re-polarisation of the Th1 / Th2 response, and modulation of dendritic cell function. The helminths down regulate the pro-inflammatory Th1 cytokines, Interleukin-12 (IL-12), Interferon-Gamma (IFN-γ) and Tumour Necrosis Factor-Alpha (TNF-ά), while promoting the production of regulatory Th2 cytokines such as IL-10, IL-4, IL-5 and IL-13.

That helminths modulate host immune response is proven, as the core assertion of the hygiene hypothesis appears to have been, with the recent publication of a study demonstrating that co-evolution with helminths has shaped at least some of the genes associated with Interleukin expression and immunological disorders, like Crohn's, ulcerative colitis and Celiac Disease. Much of the research that has been published now indicates a key role, for what have been traditionally regarded as disease causing organisms, so that their relationship to humans as hosts should not be classified as parasitic, rather as mutualistic, symbionts [7].

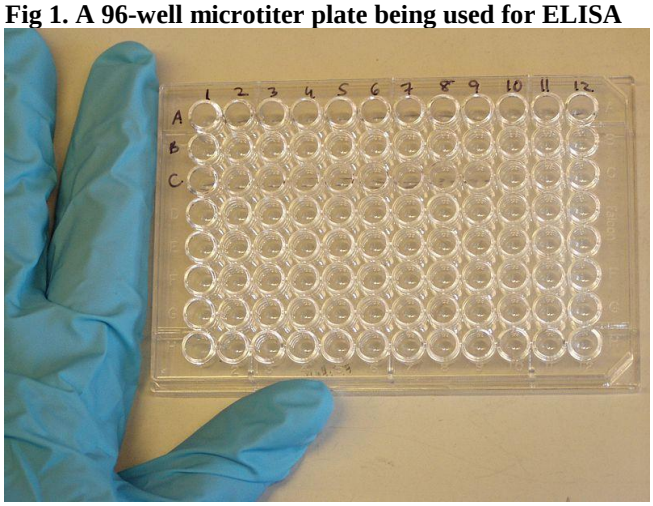
Diagnostic immunology

The specificity of the bond between antibody and antigen has made it an excellent tool in the detection of substances in a variety of diagnostic techniques. Antibodies specific for a desired antigen can be conjugated with a radiolabel, fluorescent label, or color-forming enzyme and are used as a probe to detect it. However, the similarity between some antigens can lead to false positives and other errors in such tests by antibodies cross-reacting with antigens that aren't exact matches.

ELISA

Enzyme-linked immunosorbent assay (ELISA), is a popular format of a wet-lab type analytic biochemistry assay that uses one sub-type of heterogeneous, solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample.

Fig 1. A 96-well microtiter plate being used for ELISA



The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. Attempting to detect (and quantify) the presence of the antigen in the sample proceeds as follows: Antigens from the sample are attached to a surface. Then a further specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a sandwich ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by

a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample [8].

It is worth noting that ELISA can perform other forms of ligand binding assays instead of strictly immuno assays, though the name carried the original immuno because of the common use and history of development of this method. The technique essentially requires any ligating reagent that can be immobilized on the solid phase along with a detection reagent that will bind specifically and use an enzyme to generate a signal that can be properly quantified. In between the washes only the ligand and its specific binding counterparts remain specifically bound or immunosorbed by antigen-antibody interactions to the solid phase, while the nonspecific or unbound components are washed away. Unlike other spectrophotometric wet lab assay formats where the same reaction well (e.g. a cuvette) can be reused after washing, the ELISA plates have the reaction products immunosorbed on the solid phase which is part of the plate and thus are not easily reusable.

Principle

As a wet lab analytic biochemistry assay, ELISA involves detection of an analyte (i.e. the specific substance whose presence is being quantitatively or qualitatively analyzed) in a liquid sample by a method that continues to use liquid reagents during the analysis (i.e. controlled sequence of biochemical reactions that will generate a signal which can be easily measured quantified and interpreted as a measure of the amount of analyte in the sample) that stays liquid and remains inside a reaction chamber or well that is needed to keep the reactants contained; It is opposed to dry lab that can use dry strips - and even if the sample is liquid (e.g. a measured small drop), the final detection step in dry analysis involves reading of a dried strip by methods such as reflectometry and does not need a reaction containment chamber to prevent spillover or mixing between samples.

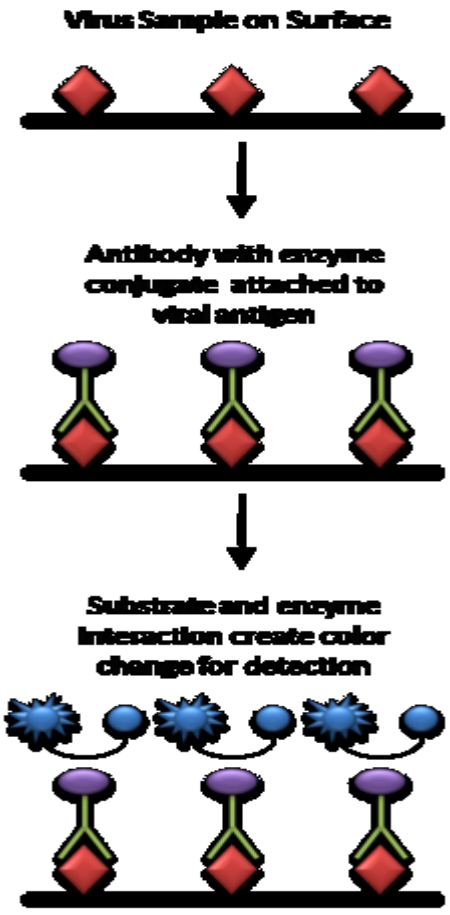
As a heterogenous assay, ELISA separates some component of the analytical reaction mixture by adsorbing certain components onto a solid phase which is physically immobilized. In ELISA a liquid sample is added onto a stationary solid phase with special binding properties and is followed by multiple liquid reagents that are sequentially added, incubated and washed followed by some optical change (e.g. color development by the product of an enzymatic reaction) in the final liquid in the well from which the quantity of the analyte is measured. The qualitative reading usually based on detection of intensity of transmitted light by spectrophotometric which involves quantitation of transmission of some specific type of light through the liquid (as well as the transparent bottom of the well in the multi-well plate format). The sensitivity of detection depends on amplification of the signal during the analytic reactions. Since enzyme reactions are very well known amplification processes, the signal is generated by enzymes which are linked to the detection reagents in fixed proportions to allow accurate quantification - thus the name Enzyme linked.

The analyte is also called the ligand because it will specifically bind or ligate to a detection reagent and thus ELISA falls under the bigger category of Ligand Binding Assays. The ligand-specific binding reagent is immobilized i.e. usually coated and dried onto the transparent bottom and sometimes also side wall of a well (the stationary solid phase'/solid substrate here as opposed to solid microparticle/beads that can be washed away), which is usually constructed as a multi-well plate known as the Elisa Plate. Conventionally, like other forms of immunoassays the specificity of Antigen-Antibody type reaction is used because it is easy to raise an antibody specifically against an antigen in bulk as a reagent. Alternatively if the analyte itself is an antibody its target antigen can be used as the binding reagent [9].

Types

Indirect ELISA

Fig 2. Indirect ELISA diagram



The steps of indirect ELISA follows the mechanism below:-

A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions. A solution of non-reacting protein, such as bovine serum albumin or casein, is added to block any plastic surface in the well that remains uncoated by the antigen.

Next the primary antibody is added, which binds specifically to the test antigen that is coating the well. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.

Afterwards, a secondary antibody is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody.

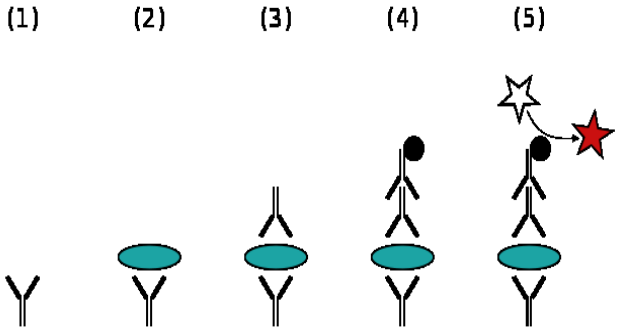
A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen. This can be helpful in a clinical setting, and in R&D. The higher the concentration of the primary antibody that was presents in the serum, the stronger the color change. Often a spectrometer is used to give quantitative values for color strength.

The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound, the enzyme molecules will produce many signal molecules. Within common-sense limitations, the enzyme can go on producing color indefinitely, but the more primary antibody is present in the donor serum the more secondary antibody + enzyme will bind, and the faster color will develop. A major disadvantage of the indirect ELISA is that the method of antigen immobilization is non-specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich or direct ELISA provides a solution to this problem, by using a capture antibody specific for the test antigen to pull it out of the serum's molecular mixture.

ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result (yes or no) for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation (error inherent in a test) is often used to distinguish positive from negative samples. In quantitative ELISA, the optical density (OD) of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule. For example, if a test sample returns an OD of 1.0, the point on the standard curve that gave OD = 1.0 must be of the same analyte concentration as your sample [10].

Sandwich ELISA

Fig 3. A sandwich ELISA. (*1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.*



A less-common variant of this technique, called sandwich ELISA, is used to detect sample antigen. The steps are as follows:

- Prepare a surface to which a known quantity of capture antibody is bound.
- Block any nonspecific binding sites on the surface.
- Apply the antigen-containing sample to the plate.
- Wash the plate, so that unbound antigen is removed.
- A specific antibody is added, and binds to antigen (hence the 'sandwich': the Ag is stuck between two antibodies);
- Apply enzyme-linked secondary antibodies as detection antibodies that also bind specifically to the antibody's Fc region (non-specific).
- Wash the plate, so that the unbound antibody-enzyme conjugates are removed.
- Apply a chemical that is converted by the enzyme into a color or fluorescent or electrochemical signal.
- Measure the absorbency or fluorescence or electrochemical signal (e.g., current) of the plate wells to determine the presence and quantity of antigen.

The image to the right includes the use of a secondary antibody conjugated to an enzyme, though, in the technical sense, this is not necessary if the primary antibody is conjugated to an enzyme. However, use of a secondary-antibody conjugate avoids the expensive process of creating enzyme-linked antibodies for every antigen one might want to detect. By using an enzyme-linked antibody that binds the Fc region of other antibodies, this same enzyme-linked antibody can be used in a variety of situations. Without the first layer of capture antibody, any proteins in the sample (including serum proteins) may competitively adsorb to the plate surface, lowering the quantity of antigen immobilized.

Use of the purified specific antibody to attach the antigen to the plastic eliminates a need to purify the antigen from complicated mixtures before the measurement, simplifying the assay, and increasing the specificity and the sensitivity of the assay. A descriptive animation of the application of sandwich ELISA to home pregnancy testing can be found here.

Competitive ELISA

A third use of ELISA is through competitive binding. The steps for this ELISA are somewhat different than the first two examples:

- Unlabeled antibody is incubated in the presence of its antigen (Sample).
- These bound antibody/antigen complexes are then added to an antigen-coated well.
- The plate is washed, so that unbound antibody is removed. (The more antigen in the sample, the less antibody will be able to bind to the antigen in the well, hence competition.)
- The secondary antibody, specific to the primary antibody is added. This second antibody is coupled to the enzyme.
- A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.
- The reaction is stopped in order to prevent eventual saturation of the signal

Note that some competitive ELISA kits include enzyme-linked antigen rather than enzyme-linked antibody. The labeled antigen competes for primary antibody binding sites with your sample antigen (unlabeled). The more antigen in the sample the less labeled antigen is retained in the well and the weaker the signal). It is common that the antigen is not first positioned in the well.

For the detection of HIV antibodies, the wells of microtiter plate are coated with the HIV antigen. Two specific antibodies are used, one conjugated with enzyme and the other present in serum (if serum is positive for the antibody). Competition occurs between the two antibodies for the same antigen. Sera to be tested are added to these wells and incubated at 37 degrees and then washed. If antibodies are present, antigen-antibody reaction occurs. There is no antigen left for the enzyme labelled specific HIV antibodies. These antibodies remain free upon addition and are washed off during washing. Substrate is added but there is no enzyme to act on it, therefore, positive result shows no color change.

A new technique uses a solid phase made up of an immunosorbent polystyrene rod with 8-12 protruding ogives. The entire device is immersed in a test tube containing the collected sample and the following steps (washing, incubation in conjugate and incubation in chromogenous) are carried out by dipping the ogives in microwells of standard microplates pre-filled with reagents [11].

The advantages of this technique are as follows:

- The ogives can each be sensitized to a different reagent, allowing the simultaneous detection of different antibodies and/or different antigens for multi-target assays;
- The sample volume can be increased to improve the test sensitivity in clinical (blood, saliva, urine), food (bulk milk, pooled eggs) and environmental (water) samples;
- One ogive is left unsensitized to measure the non-specific reactions of the sample;
- The use of laboratory supplies for dispensing sample aliquots, washing solution and reagents in microwells is not required, facilitating the development of ready-to-use lab-kits and on-site kits.

Applications

The ELISA was the first screening test widely used for HIV because of its high sensitivity. In an ELISA, a person's serum is diluted 400-fold and applied to a plate to which HIV antigens are attached. If antibodies to HIV are present in the serum, they may bind to these HIV antigens. The plate is then washed to remove all other components of the serum. A specially prepared secondary antibody — an antibody that binds to other antibodies — is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme.
Thus, the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence. ELISA results are reported as a number; the most controversial aspect of this test is determining the cut-off point between a positive and a negative result.

A cut-off point may be determined by comparing it with a known standard. If an ELISA test is used for drug screening at workplace, a cut-off concentration, 50ng/mL, for example, is established, and a sample that contains the standard concentration of analyte will be prepared. Unknowns that generate a signal that is stronger than the known sample are positive. Those that generate weaker signal are negative [12].

The ELISA test to detect various kind of diseases, such as Malaria, Chagas' disease, and Johne disease. ELISA tests also are used as in in vitro diagnostics in medical laboratories. The other uses of ELISA include:

- detection of Mycobaterium antibodies in tuberculosis.
- detection of rotavirus in feces.
- detection of hepatitis B markers in the serum.
- detection of enterotoxin of E. coli in feces.
- detection of HIV antibodies in blood samples.Evolutionary immunology

Study of the immune system in extant species is capable of giving us a key understanding of the evolution of species and the immune system. A development of complexity of the immune system can be seen from simple phagocytotic protection of single celled organisms, to

circulating antimicrobial peptides in insects to lymphoid organs in vertebrates. However, it is important to recognize that every organism living today has an immune system that has evolved to be absolutely capable of protecting it from most forms of harm; those organisms that did not adapt their immune systems to external threats are no longer around to be observed. Insects and other arthropods, while not possessing true adaptive immunity, show highly evolved systems of innate immunity, and are additionally protected from external injury (and exposure to pathogens) by their chitinous shells.

Reproductive immunology

This area of the immunology is devoted to the study of immunological aspects of the reproductive process including fetus acceptance. The term has also been used by fertility clinics to address fertility problems, recurrent miscarriages, premature deliveries, and dangerous complications such as pre-eclampsia [13].

CONCLUSION

ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool for determining serum antibody concentrations (such as with the HIV test or West Nile Virus). It has also found applications in the food industry in detecting potential food allergens such as milk, peanuts, walnuts, almonds, and eggs. ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs.

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