Immunogenicity testing of biopharmaceuticals

The detection and characterization of immune responses towards new biopharmaceuticals is essential for ensuring the clinical safety of these biological therapeutics.

Eurofins Global Central Laboratory provides a whole range of comprehensive testing capabilities to support immunogenicity assessments in clinical trials.

- Custom assay selection and or development for anti-drug antibody (ADA) detection
- Characterization of binding and neutralizing capacity of anti-drug antibodies
- Cellular immune response determination
- B cell quantification

Biological/biotechnology-derived proteins (Biopharmaceuticals) are increasingly used as therapeutic agents. It has been recognized that these proteins may induce humoral and cellular immune response. The consequences of an immune reaction to a therapeutic protein range from transient appearance of antibodies without any clinical significance to severe life threatening conditions. Potential clinical consequences are severe hypersensitivity-type reaction, decrease in efficacy and induction of autoimmunity, including antibodies to the endogenous form of the protein. It is therefore very important to select and/or develop assays & assay strategies for assessment of such immune responses. Most effort is usually focused on antibody detection and characterization. This usually includes a screening assay for identification of antibody positive samples/patients, analytical immunochemical procedures for confirming the presence of antibodies and determining antibody specificity and functional bioassay(s) for the assessment of the neutralizing capacity of antibodies [1,2].

Here we present an overview of the different assay types Eurofins Global Central Laboratory is offering to determine the degree of immunogenicity of the compound under investigation. These assays are developed in-house and validated according to SOPs based on selected literature references [3-7].

Detection of a humoral immune response towards your compound

In first instance one needs to determine whether a humoral immune response is present in a patient treated with a specific compound. In order to do so, Eurofins Global Central Laboratory offers the service of developing custom assays to detect the presence of antibodies towards your compound. These assays are all ELISA-based but can be developed using different detection methods ranging from simple colorimetric detection over fluorescent or luminescent detection to the more sensitive electrochemiluminescent detection using an MSD platform. All these assays share the common principle that a screening assay is setup that determines whether a sample is positive or not for a specific immune response. Positive samples are then confirmed using a competition assay with the drug of interest, confirming the specificity of the observed response. Not only can these assays be used for the detection of total Ig molecules but also isotype-specific (IgG, IgE or IgM) responses can be determined.

Determination of the avidity of the antibodies formed

When samples appear positive in the previous assay, it might be of great interest to obtain more information on the characteristics of the antibodies formed in the body. Therefore, we develop a titration assay in order to determine the IC50 of the antibodies present in your

sample. In this assay a dilution series of you compound is added to a certain amount of antibodies and this mixture is analyzed using the assay described in the previous section.

Determination of the neutralizing capacity of the antibodies formed

When antibodies are produced that can bind your compound, this does not per se mean that they will also inhibit the function of your compound in vivo. It is very well possible that the antibodies bind at a less important epitope of your compound and leave the active site accessible. In order to demonstrate that the antibodies formed also can inhibit the function of your compound we can setup specific bioassays. In these assays the action of your compound towards a specific cell line is investigated in the presence and absence of the patient serum sample. In case the antibodies present in the serum sample do have neutralizing capacity, a clear effect is observed in the bioassay.

Detection of a cellular immune response towards your compound

For the analysis of cellular immunogenicity, Eurofins Global Central Laboratory prepares PBMC's from whole blood samples using Ficoll sepa-ration. Cells are then stimulated with your specific compound and both surface markers (CD4 and CD8) and intracellular cytokine levels will be determined using a flow cytometer. Using this assay, the amount of cytotoxic T cells and T helper cells can be determined that respond to the compound by producing cytokines. Using this experiment it can very rapidly be determined whether a patient might potentially develop a so-called "cytokine storm".

Memory B cell quantification to your compound (B cell Elispot)

For this assay, also PBMC's will be used. The cells will be cultivated using a CO2 incubator at 37°C and are incubated in plates coated with your specific compound. The plasma B cells produce antibodies directed against your compound and form spots. These spots are then detected by an enzyme-labeled anti-human antibody and a chromogenic substrate. These spots can then be counted either manually or an automated reader.

References

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