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Current and Future Issues in Sensitisation Testing

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Sensitisation is one of the biological risks that needs to be addressed for any medical device before clearance or clinical evaluation. This article describes the state of the art for this evaluation and also new variants of the procedure to increase the accuracy of these tests, particularly when some metals, latex-based products and macromolecular proteins such as collagen are concerned.

Current weaknesses

Under some circumstances, immunity, instead of providing protection, produces damaging and sometimes fatal results. Deleterious reactions of this type are known collectively as hypersensitivity or allergic reactions. Antigens that commonly cause hypersensitivity or allergic reactions are referred to as allergens. These reactions differ from protective immune reactions in that they are exaggerated or inappropriate and damaging to the host.

Consideration of the potential to cause allergenicity must be given to any product that comes into contact with the body, whether it is a surgical drape, a contact lens or an implant, before clearance or clinical evaluation. The rationale for this is that aqueous and/or lipophilic chemicals can migrate from the device into the patient via fluids such as blood and tissue fluids. After repeated contact, a sensitisation reaction marked by redness and swelling, known as delayed contact or Type IV sensitisation, may occur. In addition, acute hypersensitivity reactions may develop for specific antigens and exposures and these can be life threatening; these are often called anaphylactic or Type I reactions.

This article discusses the delayed hypersensitivity contact evaluation that is required by ISO 10993 Biological Evaluation of Medical Devices, Part 10, Tests for Irritation and Sensitisation.[1] Of course, hypersensitivity is only one of the potential immunotoxic effects, which include chronic inflammation, immunosuppression, immunostimulation and autoimmunity.[2] To examine other potential immunotoxic effects, readers should consult the Food and Drug Administration Immunotoxicity Testing Guidance (see Table I).[2]

Basis of allergic reactions

Hypersensitisation reflects an increased reactivity to an antigen to which a person has been previously exposed. Type I and Type IV reactions are the most common and Type I reactions are the most serious. Type II and Type III reactions involve antibodies (IgG or IgM but not IgE) and complement, but are relatively rare

Table I: Potential immunotoxic effects of materials (FDA Guidance 1999).[2]

	Immunotoxic effects				
	Hypersensitivity	**Chronic inflammation**	**Immuno-suppression**	**Immuno-stimulation**	**Auto-immunity**
Polymers, plastics	X	X		X	
Metals	X	X	X	X	X
Ceramics, glasses, composites		X			
Biological materials	X	X	X	X	X
Others	X	X	X	X	X

and are less likely to occur with medical devices or materials.

Type I: These anaphylactic reactions are mediated by IgE antibodies, which bind through the Fc portion to receptors on mast cells and basophils. When crosslinked by antigens, the IgE antibodies trigger the mast cells and basophils to release pharmacologically active agents that are responsible for the characteristic symptoms of anaphylaxis. Reactions are rapid, occurring within minutes after reexposure to antigen.

Type IV: Cell-mediated immunity reaction, also called delayed-type hypersensitivity or the tuberculin reaction, is mediated by T cells rather than by antibodies. On activation, the T cells release lymphokines that cause accumulation and activation of macrophages, which, in turn, cause local damage. This type of reaction has a delayed onset and occurs 1–2 days after challenge with antigen.

Antigen-presenting cells are critical in initiating and controlling allergic inflammation. Among them dendritic cells and cutaneous Langerhans cells are particularly important because they secrete IL12 and present antigen to CD4+ T cells. As a consequence of activation by antigen, CD4+ T cells secrete cytokine soluble factors that affect T cells, B cells and many other cell types. Subsets of CD4+ T cells have been defined by the range of cytokines they produce:

- Th1 cells secrete IL2, IL12 and interferon, which activate other T cells, NK cells and macrophages. They represent the "cellular" immune response involved in Type IV hypersensitivity.
- Th2 cells secrete, among other lymphokines, IL4, which induces isotypic commutation of B-lymphocytes to synthetise IgE. This reaction is characterised by high levels of IgE, which do not bind the complement, corresponding to the "humoral" response or immediate Type I hypersensitivity.

Evaluating sensitising potential

Sensitisation methods described in ISO 10993, Part 10, address only delayed-type hypersensitisation (Type IV), which is only part of the immunology requirements for some devices. The maximisation test described by Magnusson and Kligman, which has been modified for use with polar and nonpolar extracts, is recognised as the best and the most precise tool available for the nonclinical study of sensitisation.[3] It is achieved in guinea pigs and is generally called the Guinea Pig Maximisation Test (GPMT).

Typically, a minimum of 10 guinea pigs are used for each test extract. Negative control evaluations are performed using five animals exposed to each of the extraction vehicle (without material). Additional animals may be used to determine the proper concentration of test material to use or to dose positive control animals.

For phase I induction, injections are given intradermally with and without Freund's complete adjuvant. One week later the second introduction of the test agent is performed topically over the injection site. During the rest period (2 weeks) cellular changes occur and immunity develops. The animals are challenged by patch test 2 weeks later. Each site is scored for erythema and oedema 1 h after removing the patches and at 24 h and 48 h after patch removal. The rate of allergenicity of the test material is noted according to Table II.

A procedure to increase accuracy

The results of positive maximisation testing in the guinea pig are predictive for humans. However, it cannot be assumed that a negative response in a maximisation test will carry over to a larger human population. Human allergens generally sensitise guinea pigs although it is the consensus that the guinea pig is less sensitisable than the human. The key difficulty is that some substances known to be human sensitisers have failed to sensitise the guinea pig. These include some metals, drugs, latex-based products and macromolecular proteins such as collagen.

To increase the accuracy of the GPMT, the maximisation procedure has been modified in the following way.

- The second induction is achieved by combining intradermal and topical exposure.
- The challenge phase, after 2 weeks (day 21) is performed by topical exposure followed by intradermal injection by day 23. Results of intradermal exposures and topical exposures are always analysed separately, but by using both approaches even substances considered as weak antigens in the GPMT could be detected.

It cannot be assumed that a negative response in a maximisation test will carry over to a larger human population.

A comparative evaluation using this combined technique and the GPMT is given in Table III. The first column reports the sensitisation observed in the GPMT test. The second column reports a newly developed test (Test 2) where the challenge combines topical (day 21) and intradermal administration (day 23). The third column shows (Test 3) the newly combined topical and intradermal administration at the three steps of the stimulation: induction I (day 0), induction II (day 8–9) and challenge (day 21).

Table III shows that several macromolecular components known for their capacity to induce delayed-type hypersensitivity (DTH) in humans were not detected in the GPMT whereas Test 2 and particularly Test 3 identified them as sensitisers. One "treated" latex did not exhibit this risk because of adequate processing of the latex.

These findings suggest that large molecular weight components may not always be able to reach antigen-presenting cells located in the dermis/epidermis. With regard to latex-containing devices, it is well established that latex proteins are associated with Type I immediate hypersensitivity, which is not detected by the GPMT (see Test 2), and that chemical additives such as thiurams and carbamates are responsible for the delayed Type IV hypersensitivity

detected in Test 3.

For chemicals that cannot be administered by the intradermal route, including nonresorbing materials, pastes or necrogenic materials, occlusive topical applications with or without intradermal injection of Freund's adjuvant can be used (Buehler test).[4]

Additional undetected hazards

Some devices can also induce systemic anaphylactic reactions. These reactions are seen with collagen, which is used to coat devices such as vascular prostheses or injected into dermal or tissular implants and wound dressings, and with latex from gloves and other devices. This hazard is not explored by the GPMT and is not addressed by ISO 10993, Part 10. To induce a systemic anaphylactic reaction, guinea pigs are sensitised by one or several injections of the test substance. Freund's complete adjuvant is inappropriate to enhance sensitisation, whereas aluminium hydroxide or killed bacteria are potent inducers of IgE production.

Few models have been developed, but by combining systemic and local anaphylaxis, the potential to induce immediate hypersensitivity reactions can be detected as far as large molecular weight molecules are concerned.[5] As shown in Table III, the GPMT-modified test was able to detect Type I immediate sensitivity reactions by the intradermal challenge or by an additional intravenous challenge.

Changes in the standard

ISO 10993, Part 10, is currently under review and a tiered approach for the GPMT has been proposed. Each extract will start with 10 test and 5 control animals each. If the results are clear (delayed sensitiser, nonsensitiser), the study may stop. If the results are equivocal, a rechallenge should be done. If the results are not definitive, the study is to be repeated in double the number of animals for the extract involved. This approach allows Part 10 to remain in compliance with the Organisation for Economic Coopera-tion and Development's protocol.[6] In those few cases where delayed sensitisation is tested by patch test, the doses proposed will be 3 per week for 3 weeks, then rest and challenge. An ISO meeting being held in Berlin, Germany, on 25–27 September 2001, will discuss the adoption of this procedure in Part 10.

Other innovations in testing

Other trends in innovative test methods include the local lymph node assay (LLNA), which has been proposed as an alternative to the Magnusson and Kligman GPMT. The LLNA has been validated for pharmaceutical and agricultural chemicals. Selected chemical solutions are applied topically to the ears of mice. Sensitising chemicals stimulate lymphocyte activity in nearby lymph nodes. A radiolabelled compound is injected intravenously and taken up rapidly by lymph nodes if there is lymphocyte activity stimulated by a sensitiser. Quantitative results are generated by measuring the concentration of radioactivity. Currently, only a couple of laboratories have validated the LLNA method for use on device extracts. The turnaround time is 3 to 4 weeks compared with the GPMT, which requires 6 to 8 weeks or longer if rechallenge is necessary.

In addition to hypersensitivity reactions, a biomaterial may elicit autoimmune responses. For example, a foreign protein may induce IgG or IgM antibodies that cross-react with a human protein and cause tissue damage by activating the complement system. To examine other potential immunotoxic effects that may be associated with different medical device materials it is essential to refer to the FDA guidance document.[2]

Table II: Rating of sensitisation response* using ASTM F 720-81.

% Sensitised animals	Grades	Classification
0	0	No sensitising
1 to 10	I	Slight
11 to 30	II	Mild
31 to 60	III	Moderate
61 to 80	IV	Strong
81 to 100	V	Extreme

* Modified classification to discriminate between nonsensitising products (Grade 0) and weak sensitising products (Grade I).

References

1. ISO 10993, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Sensitisation.
2. Guidance for Industry and FDA Reviewers: Immunotoxicity Testing Guidance, issued 6 May 1999.
3. B. Magnusson and A. Kligman, "The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximisation Test," *J. Invest. Dermatol.*, **52,** 3, 268–276 (1969).

Table III: Comparison of ISO 10993, Part10, and newly developed protocols.

Routes of exposure at days 0, 9 and 21–23	Number of sensitised animals out of total number of exposed animals		
	Test 1 GPMT ID/topical exposure/ topical exposure	Test 2 ID/topical exposure / topical exposure + ID	Test 3 ID/ID + topical exposure / ID + topical exposure
Latex bandages	0/10	4/9 sensitised animals	9/10
Latex product	0/10	—	10/10
Treated latex	0/10	—	0/10
Latex gloves	0/10	—	10/10
Vegetal protein	0/10	8/8 DTH + anaphylactic shock (IV: 2/2)	—
Animal protein 1	0/10	—	10/10
Animal protein 2	0/5	—	2/5 DTH + anaphylactic shock

ID: intradermal administration, DTH: Delayed-type hypersensitisation, IV: intravenous administration

Table IV: Glossary of terms.

Adjuvant	A substance, given with antigen, that enhances the response to the injected antigen.
Allergen	An antigen responsible for producing allergic reactions by inducing IgE synthesis.
Allergy	A term covering immune reactions to nonpathogenic antigens, which lead to inflammation and deleterious effects in the host.
Anaphylaxis	Immediate hypersensitivity response to antigenic challenge, mediated by IgE and mast cells. It is a life-threatening allergic reaction caused by the release of pharmacologically active agents.
Antibody	Serum protein formed in response to immunisation; antibodies are generally defined in terms of their specific binding to the immunising antigen.
Antigen	Any foreign material that is specifically bound by antibody or lymphocytes; also used to describe materials used for immunisation. Compare with immunogen.
Antigen-presenting cell (APC)	A specialised type of cell, bearing cell-surface Class II major histocompatibility complex (MHC), molecules, involved in processing and presentation of antigen to inducer T cells.
B lymphocyte (B cell)	The precursors of antibody-forming plasma cell; this cell expresses immunoglobulin on its surface.
Cell-mediated immunity (CMI)	Immune reaction mediated by T cells; in contrast to humoral immunity, which is antibody-mediated; also referred to as delayed-type hypersensitivity.
Complement	A series of serum proteins involved in the mediation of immune reactions. The complement cascade is triggered classically by the interaction of antibody with specific antigen.
Cytokines	Soluble substances secreted by cells, which have a variety of effects on other cells.
Delayed-type hypersensitivity (DTH)	A T cell-mediated reaction to antigen, which takes 24–48 h to develop fully and involves release of lymphokines and recruitment of monocytes and macrophages; also called cell-mediated immunity.
Freund's complete adjuvant	An oil containing killed mycobacteria and an emulsifier which, when emulsified with an immunogen in aqueous solution, enhances the immune response to that immunogen following injection. Termed "incomplete" Freund's adjuvant if mycobacteria are not included.
GPMT	Magnusson Kligman Guinea Pig Maximisation Test.
Humoral immunity	Any immune reaction that can be transferred with immune serum (as opposed to cell-mediated immunity). In general, this term refers to resistance that results from the presence of specific antibody in the serum.
Hypersensitivity	State of reactivity to antigen that is greater than normal for the antigenic challenge; hypersensitivity is the same as allergy and denotes a deleterious outcome rather than a protective one.
Immediate-type hypersensitivity	Hypersensitivity tissue reaction occurring within minutes after the interaction of antigen and antibody.
Immunogen	A substance capable of inducing an immune response (as well as reacting with the products of an immune response). Compare with antigen.
Immunoglobulin (Ig)	A general term for all antibody molecules. Each Ig unit is made up of two heavy chains and two light chains and has two antigen-binding sites (IgA, IgG, IgE, IgM).
Interferon (IFN)	Proteins synthetised released by activated T-lymphocytes (IL1, IL2, IL4).
Interleukins	Glycoproteins secreted by a variety of leukocytes that have effects on other leukocytes.
Killer T cell	A T cell capable of specifically killing a target cell expressing foreign antigen bound to MHC molecules on the surface of the target cell; also called cytotoxic T cell.
Lymphocyte	Small cell with virtually no cytoplasm, found in blood, in all tissue, and in lymphoid organs such as lymph nodes, spleen and Peyer's patches, and bears antigen-specific receptors.
Lymphokines	Soluble substances secreted by lymphocytes, which have a variety of effects on lymphocytes and other cell types. Cytokines are secreted by lymphocytes.
Macrophage	A large phagocytic cell of the mononuclear series.
T-lymphocyte	A lymphocyte that differentiates in the thymus.

4. E.V. Buehler, "Delayed Contact Hypersensitivity in the Guinea Pig," *Arch. Dermatol.*, **91,** 171–175 (1965).
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