

Chapter 1

The Use of T Cells in Hazard Characterization of Chemical and Drug Allergens and Integration in Testing Strategies

Foreword

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Abbreviations

APC	Antigen-presenting cell
CTLA-4	Cytotoxic T lymphocyte antigen 4
DC	Dendritic cells
IFN- γ	Interferon-gamma
LLNA	Local lymph node assay
MHC	Major histocompatibility complex
p-i concept	Pharmacological interaction concept
pMHC	Peptide major histocompatibility complex
TCR	T-cell receptor
TH1	T helper 1 lymphocyte
TH2	T helper 2 lymphocyte
Treg	T regulatory lymphocyte

1.1 General Introduction on the Role of T Lymphocytes

The activation of T lymphocytes expressing the $\alpha\beta$ T-cell receptor (TCR) is a crucial event in the development of adaptive immune responses. T lymphocytes circulate throughout the organism and enter sequentially into contact with

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antigen-presenting cells (APC) displaying on their surface antigenic peptides bound to molecules of the major histocompatibility complex (MHC). The binding of TCR to specific peptide-MHC complexes (pMHC) triggers a cascade of intracellular signaling effectors leading to T-cell cytokine production, proliferation, and/or killing of target cells (Valitutti et al. 2010). However, for chemicals and drugs, the concept of antigenic structures presented to T cells by APC can vary compared to what is described for “classical” antigens. A chemical or a drug is not antigenic on its own because of its small size and it must first bind to a high molecular weight protein to be immunogenic. A stable covalent binding of a chemically reactive drug or chemical to a larger protein or peptide is then immunogenic and can be recognized directly by T cells after undergoing antigen processing of the hapten-carrier complex (hapten hypothesis). The chemical properties of hapten-like drugs are then crucial for the generation of antigenic epitopes and activation of the innate immune system. However, this is only one possibility for T-cell activation by chemicals, and other situations have been also described such as direct binding of chemicals to peptide loaded with HLA molecules, the p-i concept proposing that a drug is able to stimulate T cells directly without forming a hapten in a HLA-dependent manner, and the specific case of transition metals such as nickel forming coordination liaisons with HLA-loaded peptides and the TCR (Vocanson et al. 2009; Yun et al. 2012). These aspects will be addressed in this book by Christine Louis-Dit-Sully and Wolfgang W. A. Schamel in the chapter *Activation of the TCR Complex by Small Chemical Compounds* and also by H.-U. Weltzien, J.-F. Nicolas, and S. F. Martin in the chapter *T Cell Responses to Contact Allergens*. Identification of the haptenized peptides presented to T cells from circulating drug-bound proteins such as albumin found in allergic patients using specific methodologies such as mass spectrometry is also a very valuable approach to understand the mechanism underlying drug allergy, and this aspect is covered by C. J. Earnshaw, T. Pecaric-Petkovic, B. K. Park, and D. J. Naisbitt in the chapter *T-Cell Responses to Drugs and Drug Metabolites* in this book.

T cells are highly motile and scan most of the body spaces in search for rare APC presenting the antigens that will trigger their activation. Once the T-cell/APC cognate interaction is established, the spatial frame is restricted to the cellular interface, named the immunological synapse (Valitutti et al. 2010). At this specialized contact area, the TCR and multiple accessory molecules are simultaneously engaged, leading to the activation of varied and interconnected signaling pathways. These profound changes in spatial organization are instrumental for signaling in T cells and, most importantly, create a privileged site for cell–cell cooperation, allowing selectivity of T-cell responses. The time frame of T-cell activation encompasses molecular events happening within seconds and sustained for hours or days up to the acquisition of functional properties. Time plays a key role in the process of T-cell activation. The duration of TCR-mediated signal transduction in individual T cells (occurring on the scale of hours) and the duration of TCR/pMHC binding half-lives (on the scale of seconds) are crucial parameters in T-cell activation. Moreover, depending on the activation state of responding T cells, the quality of the presented antigen, the context of the antigenic stimulation, as well as duration

and “rhythm” of productive TCR signaling can profoundly influence activation and effector functions in T cells. Indeed, modification of the cellular microenvironment by chemicals can also influence and dictate the phenotype of DC and the type of immune response. When testing for T-cell activation by chemicals and drugs in vitro and according to what is known on T-cell biology and DC-T-cell interaction, the likely basis of an experimental model should be a DC-T-cell coculture model measuring T-cell activation parameters, T-cell proliferation, and cytokines/chemokines production. This paradigm is well exemplified in the chapter *Human T-Cell Priming Assay* described in this book but also in the chapter *Regulatory Role of Programmed Death-Ligand 1 in Dendritic Cell-T Helper Coculture Assays for Detection of Contact Allergy* by M. Peiser, M. Hitzler, and A. Luch. However, stimulating naïve T cells in vitro with chemicals is still a scientific and technical challenge.

The naïve T-cell repertoire is extraordinarily diverse because it contains an enormous number of distinct T-cell clones, each represented by only a few cells (Arstila et al. 1999). This repertoire is mainly formed by positive and negative selections in the thymus leading to the generation of functional CD4⁺ T cells able to recognize foreign peptides presented by HLA class II molecules. Upon antigenic stimulation in secondary lymphoid organs, rare antigen-specific naïve T cells undergo clonal expansion and differentiate to effector and memory cells. Thus, the memory T-cell repertoire contains a collection of expanded T-cell clones that reflect the antigenic experience of the individual. Molecular studies indeed established that the TCR diversity is at least 100-fold lower in the memory compared with the naïve repertoire. The identification and characterization of antigen-specific T cells in the naïve repertoire is of fundamental relevance to understanding the process of clonal selection and of practical relevance to predicting the immunogenicity of vaccines and therapeutic proteins. In chemical and drug allergy, it is tempting to speculate that the frequency of chemical- or drug-specific naïve T cells present in a predisposed individual could be correlated to the strength of the T-cell response during the sensitization process (Nhim et al. 2013). However, due to the artificial nature of chemical T-cell epitopes, it is not clear whether selection of chemical-specific T cells is a common phenomenon or remains limited to few donors. Selection of chemical-specific T cells could be a relatively rare event accounting for the low occurrence of chemical allergy. On the other hand, a large T-cell repertoire found in multiple donors would underline the potential of chemicals to be recognized by many donors. These points are well described by S. F. Martin, S. S. Schmucker, and A. Richter in the *Tools and Methods for Identification and Analysis of Rare Antigen-Specific T Lymphocytes* chapter and by P. R. Esser, I. Kimber, and S. F. Martin in the *Human T-Cell Priming Assay. Correlation of Contact Sensitizer Potency with T-Cell Frequency and TCR Repertoire Diversity* chapter.

Regulatory T (Treg) cells function to maintain immune tolerance preventing autoimmune diseases and unwanted immune reaction to innocuous antigens and also prevent inflammatory diseases. Regulatory CD4⁺ T cells can be divided into two main subsets, natural Tregs (nTregs) and induced Tregs (iTregs), which develop in the thymus and the periphery, respectively (Campbell and Koch 2011).

Via the TCR, activated Tregs can exert non-antigen-specific bystander suppression of other T cells. Tregs have been shown to require cell-to-cell contact to suppress target cells in vitro. Binding of CTLA-4 on Tregs to CD80 and CD86 on effector T cells, infusion of cyclic AMP through gap junctions, or direct cytolytic mechanisms have been reported to be involved in Treg-mediated suppression. In addition, Treg expression of Neuropilin-1 facilitates cell contact-mediated suppression of DC. iTregs are generated from naïve CD4⁺ T cells in the periphery, where they acquire suppressive capacity. Data from several studies support the notion that iTregs are as important as nTregs in peripheral tolerance. iTregs are also important as they have been identified in situ and positively correlate with a clinical improvement of allergy symptoms. When developing in vitro assays to assess T-cell function in response to chemical allergens, it has been shown that deleting Tregs when initiating the assay may improve its sensitivity. M. Vocanson, A. Achachi, V. Mutez, M. Cluzel-Tailhardat, B. Levarlet, A. Rozières, P. Fournier, and J.-F. Nicolas have developed the human T-cell priming assay using these observations in the chapter *Human T-Cell Priming Assay. Depletion of Peripheral Blood Lymphocytes in CD25⁺ Cells Improves the In Vitro Detection of Weak Allergen-Specific T Cells*.

The differentiation of the CD4⁺ T-cell lineage into effector cells underlies successful adaptive immune responses aimed at distinct categories of pathogens. Their functional specialization is coordinated by genetic programs that use different transcription factors to direct expression of distinct soluble mediators and surface molecules that support interactions with other immune cells (Murphy and Stockinger 2010). The first paradigm for this functional diversification was the description of TH1 and TH2 CD4⁺ effector subsets by Mosmann and Coffman in 1986. TH1 cells were thought to be responsible for delayed-type hypersensitivity, activating macrophages through release of interferon (IFN)- γ and enabling them to kill intracellular pathogens. TH2 cells were considered the classical helper T cells providing help to B cells to generate class-switched antibodies. In the case of beta-lactam allergy, M. J. Torres Jaén, C. Mayorga, N. Blanca-López, and M. Blanca Gómez are showing that T cells are key factor in all types of hypersensitivity reactions to beta-lactams, regulating both IgE production or acting as effector cells, with a different profile of cytokine production. Indeed, in the chapter *Hypersensitivity Reactions to Beta-Lactams*, they described that a TH1 pattern is observed in non-immediate reactions to beta-lactams, whereas a TH2 pattern is expressed in CD4⁺ T cells in immediate reactions that are mostly IgE mediated.

1.2 How to Integrate In Vitro Tests Using T Cells in a Testing Strategy?

An excellent working definition of allergy is the adverse health effects that may result from the stimulation of an immune response.

For the practicing toxicologist, the main issues are chemical, protein and drug allergy, and the need to identify and characterize hazards and to develop effective risk assessment and risk management strategies.

Allergy develops in two phases. In the first phase, exposure to an allergen (at sufficient levels and via a relevant route of exposure) causes immunological priming and the acquisition of sensitization. If subsequently the now sensitized (primed) subject is exposed to the same material (again by a relevant route of exposure), then an allergic reaction can be elicited.

A key objective is, therefore, to have available methods that support the identification and characterization of materials (in the context of this book small molecular weight chemicals and drugs) that have the potential to induce the development of sensitization.

Historically animal models have been used. These have proved effective for the safety assessment of skin-sensitizing chemicals but less so for chemicals that cause sensitization of the respiratory tract and drug allergy. More recently, however, the scientific landscape has changed, and for various reasons there is an appetite (and in some instances a regulatory need) to develop alternative methods that will support effective safety assessments without a requirement for animal models.

The development of effective nonanimal methods for hazard identification has proven to be a significant challenge, and despite a very substantial and sustained investment, there are currently no fully validated alternative methods available (although the situation may change in the next 12 months). A greater challenge still is designing alternative test methods that, in addition to providing an accurate identification of hazard, also give an estimate of relative potency (an important first step in the risk assessment process).

Many strategies for alternative test method development have been described, and although attention to date has focused largely, although not exclusively, on skin sensitization, it is clear that success in that area would pave the way for innovations in the assessment of the potential for sensitization of the respiratory tract and drug allergy.

With the overall objective of designing a method, or a panel of methods, that will provide a sound basis for safety assessment, the aim is to model *in vitro* or *in silico* pivotal events or processes that are required for the effective acquisition of sensitization. Using skin sensitization as an example, it is possible to identify a variety of critical steps along the biological pathway to sensitization, and these include (a) the ability of chemicals to gain access to the viable epidermis; (b) the native or acquired ability of chemicals to form stable associations with macromolecules to generate an immunogenic complex; (c) the triggering by chemicals of danger signals and the innate immune system as a prelude to the initiation of an adaptive immune response; (d) the activation, mobilization, and maturation of DC populations that are able to process, deliver, and present antigen to the adaptive immune system; and (e) the elicitation of T lymphocyte responses of the necessary vigor and quality to induce immunological priming and the acquisition of sensitization.

In each of the above areas, *in vitro* and/or *in silico* approaches have been proposed. It is beyond the scope of this short introduction to provide a survey of

the various experimental systems that have been developed. Rather, the focus here is on the last of these approaches and the activation by allergen of T lymphocyte responses.

T lymphocyte activation (and subsequent clonal expansion and functional differentiation) is the hallmark of sensitization. Thus, for instance, the animal assay that is most commonly used for the assessment of skin-sensitizing potential (the mouse local lymph node assay LLNA) has as an endpoint the proliferation of lymphocytes in lymph nodes draining the site of exposure to a chemical. This works well, *in vivo*, and it has been shown that the vigor of proliferative responses in regional lymph nodes correlates causally with the acquisition of sensitization.

Translating this to an *in vitro* approach is, however, a challenge and the subject of this book.

It is true that it is possible to measure antigen-/allergen-induced T lymphocyte responses *in vitro* when the responder cells are taken from the peripheral blood of a subject that is already primed immunologically. Such responses are facilitated by the fact that prior immunological priming has caused a selective clonal expansion of antigen-reactive lymphocytes. That is, there has already been *in vivo* a quantitative increase in the number of cells that are able to respond to the priming antigen. For this reason there are sufficient numbers of responsive cells to allow discernment of antigen-induced proliferation *in vitro*.

Such recall proliferative responses by lymphocytes *in vitro* (frequently referred to as lymphocyte transformation tests) require that expanded cells are available from a primed donor. In the context of developing alternative approaches to skin sensitization testing based on T lymphocyte responses *in vitro*, it is, of course, not possible to use animals (or humans) exposed previously to the test chemical. The need, therefore, is to develop experimental strategies that will permit the elicitation by chemicals of primary responses by naïve T lymphocytes, that is, responses by T lymphocytes that have not previously been primed and expanded in response to the relevant chemical allergen. This is a significant technical challenge, and addressing that challenge is the major focus of this book.

Given the scale of the technical problems associated with measuring naïve T-cell responses to chemical allergens (and in particular ensuring an appropriate signal to noise ratio), it could be argued that other experimental approaches may be more attractive and certainly more tractable. In answer, however, there are two strong reasons why it is appropriate to persevere with approaches based on measurement of T lymphocyte responses *in vitro*.

The first is that, as indicated above, the T lymphocyte response to allergen is the hallmark of sensitization. The other reason is this. For effective hazard characterization, it is important to understand the relative sensitizing potency of an allergen, and indeed an appreciation of potency is a key requirement for effective risk assessment (Kimber et al. 2012). The LLNA has been shown to provide a basis not only for the identification of skin-sensitizing hazards but also for the measurement of relative potency. This is because the vigor of T lymphocyte proliferation in draining lymph nodes correlates causally and quantitatively with the development of sensitization, and because of this proven quantitative relationship, it is possible to

derive from the LLNA an accurate assessment of sensitizing potency. The expectation is, therefore, that if primary T lymphocyte responses can be measured accurately *in vitro*, then it might be possible not only to identify potential hazards, but also to support effective risk assessment through provision of information on sensitizing potency.

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