Journal of Immunological Methods 495 (2021) 113072

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Future perspectives on *in-vitro* diagnosis of drug allergy by the lymphocyte transformation test

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ARTICLE INFO

Keywords:
Drug allergy
Lymphocyte transformation test (LTT)
Future perspectives *in vitro* diagnostics
T cell activation and proliferation
Cell sorting by immunomagnetic cell separation and flow cytometry
Omics by transcriptomics proteomics and metabolomics

ABSTRACT

This article aims to envisage future perspectives of the lymphocyte transformation test (LTT). We describe the select innovative techniques, which can be integrated at different stages of the LTT to potentially improve the sensitivity, specificity, or practicability of the LTT. We first focus upon the cell sorting techniques comprising immunomagnetic cell separation and flow cytometry, which can be implemented prior and after the LTT culturing step to concentrate and quantify specific immune cell types. Further, we elaborate upon three important omics techniques such as transcriptomics, proteomics, and metabolomics, which can be integrated downstream of the LTT to analyze molecular changes in specific immune cells following drug induced activation and proliferation. We also develop *visions,* how state of the art techniques used in other scientific fields, can be transferred and applied in the context of *in-vitro* detection of drug allergy.

1. Introduction

The classical lymphocyte transformation test (LTT) offers valuable information on the diagnosis of hypersensitivity caused by the specific drug. However, the limited sensitivity and specificity of the LTT still warrants a joint approach of LTT combined with the classification of the clinical phenotype of the reaction, medical history, and complementary *in-vivo* testing *i.e.* skin tests and if necessary drug provocation test *etc.* for confirming the diagnosis (Brockow et al., 2015; Mayorga et al., 2017). Despite these drawbacks and associated technical difficulties, the LTT remains an acceptable choice in clinics for diagnosing delayed type drug allergy (Karami et al., 2016; Pichler & Tilch, 2004). However, it is not amenable to routine use in standard clinics due to involvement of radioactive isotopes in the classical LTT procedure (for more information on the LTT, please see the separate article in this 'Special Issue on the *in-vitro* detection of drug allergy': "Lymphocyte transformation test: history and current approaches" by Sachs et al). Moreover, little has been changed in the basic LTT workflow (also referred to as LTT platform) since its advent in the 1960s (Huber, Huber, & Braunsteiner, 1966; Pichler & Tilch, 2004; Sarkany, 1967). Some amendments to the classical LTT have been introduced in the form of alternative readout parameters and methods. For instance, in the context of the classical LTT, the detection of activated T cells by flow cytometry or consequently produced cytokines analysis by ELISA are few of these modifications, which have already been implemented to detect drug-specific T cell activation in drug allergy (Martin et al., 2010; Sachs et al., 2002; Suthumchai et al., 2018). Additionally, researchers have modified the LTT to improve the drug specific activation through addition of IL2 (Ikeda et al., 1998), IL-7/IL-15 (Porebski et al., 2013), professional antigen presenting cells (Antunez et al., 2011; Lopez et al., 2009) or by removal of regulatory T cells (CD3+ CD25) (Srinoulprasert & Pichler, 2014). Likewise, modification of the LTT by addition of anti-CD3/anti-CD28 monoclonal antibodies has been also tried, which led to increased sensitivity but simultaneously reduced specificity (Trautmann et al., 2014). In case of certain reactive drugs, hapten mediated immune activation is an important aspect of the immunopathology (Levine & Ovary, 1961; Naisbitt et al., 2002; Schnyder & Pichler, 2009). Therefore, addition of the reactive metabolite instead of the inert drug or the

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[1] The information and views set out in this manuscript are those of the authors and do not necessarily reflect the official opinion of the Federal Institute for Drugs and Medical Devices.

https://doi.org/10.1016/j.jim.2021.113072
Received 22 December 2020; Received in revised form 22 April 2021; Accepted 12 May 2021
Available online 14 May 2021

addition of liver microsomes to generate the reactive metabolite has been also implemented (Sachs et al., 2001; Chipinda, Hettick, & Siegel, 2011; Cho & Uetrecht, 2017; for additional information please see the separate article in this 'Special Issue on the *in-vitro* detection of drug allergy': "Lymphocyte transformation test: history and current approaches" by Sachs et al). Despite of all these proposed and implemented modifications, it appears that none of the aforementioned modifications has turned out to be a 'game-changer' and substantially improved sensitivity, specificity or applicability of the LTT. Thus, there is still a room and necessity for translating and integrating innovative methods to improve the *in-vitro* diagnostics of drug allergy *via* the LTT platform.

Scientific and technical innovations in the last 20 years have transformed the contemporary laboratory assays. It has led to increased sensitivity in analyzing subtle molecular changes in diverse biological processes. As the basic mechanism of drug allergy has its roots in the activation and expansion of drug-specific memory T cells, the detection of corresponding molecular changes in the immune cells could be promising for the *in-vitro* detection of drug allergy.

The immunological basis of T cell mediated drug allergy is addressed in the separate manuscript "Lymphocyte transformation test: history and current approaches" by Sachs et al. in this "Special Issue on the vitro detection of drug allergy" (Fig. 1). Accordingly, multiple mechanisms such as haptenation, pharmacological interaction with immune receptors, or the altered peptide repertoire, have been also proposed to explain the observed T cell activation and formation of drug-specific memory T cells and are detailed elsewhere (Pichler, 2019; Weltzien et al., 1996; Yun et al., 2016). Although out-of-scope of this article, these or similar mechanisms may underlie the observed activation and proliferation of the drug-specific memory T cells in the *in-vitro* LTT platform following co-incubation of the patient's peripheral blood mononuclear cells (PBMCs) with the suspected drug. It is also important to note that the observed immune response in the *in-vitro* LTT platform may differ from the *in-vivo* situation due to limitations of formation or presentation of drug-protein conjugates *in-vitro* (Chipinda et al., 2011). However, although different types of drug allergies (*i.e.* type I – IV) may follow different mechanisms or exhibit different distal effector responses, they entail a basic mechanism of drug-mediated activation and proliferation of memory T cells during the allergic immune response (Cornejo-Garcia et al., 2007; Luque et al., 2008). These steps of the T cell activation and proliferation process are associated with changes in terms of cell numbers, transcribed genes, translated proteins, reprogrammed pathways, and thus by altered cellular metabolites[2] levels. Therefore, the analysis of activated T cells or there in induced genes, proteins, and metabolites would not only extend our understanding of the mechanisms of drug allergy but also identify potential biomarkers for monitoring drug induced immune system activation in drug allergy. In the next section, we will look at the future perspectives offered by selected innovative technologies to analyze cellular and molecular changes in the drug allergic reactions.

2. Cell isolation and sorting: immunomagnetic cell separation and flow cytometry

The classical LTT is performed using PBMCs which comprises mainly lymphocytes (B, T, and Natural killer cells), and monocytes. Upon culprit drug incubation, memory T cell activation results in cell proliferation and differentiation. However, the final classical LTT read-out parameter (stimulation index) does not differentiate exactly which cells are activated and proliferating. This adds uncertainty to the classical LTT read-out parameter of 'proliferation' because, as stated above, PBMCs are not a homogeneous mixture of cells and may contain cells which are already proliferating or may proliferate irrespective of a drug-specific activation. On another aspect, the number of drug-specific T cells are miniscule compared to the whole PBMCs population (Kalish et al., 1994; Beeler et al., 2006). Thus, even upon proliferation, the net number of the drug-specific T cells will still be low and corresponding cellular changes will be difficult to detect in the background of the whole T cell population. This may dilute any drug-specific response in the LTT 'proliferation' read-out and result in low sensitivity of the overall downstream differential gene, protein, or metabolite analysis.

The isolation and concentration of specific immune cells from PBMCs or blood using immunomagnetic cell separation technique is promising in the LTT platform, as they could increase the sensitivity and specificity of the drug specific T cell proliferation analysis by minimizing unnecessary cellular 'background noise'. The strategy of immunomagnetic cell separation could be applied to concentrate specific T cells (either naïve or memory T helper (Th) or cytotoxic T (Tc) cells) or specific antigen presenting cell (APC) population *via* positive or preferably negative selection (Horgan, Shaw, & Boirivant, 2009; Sutermaster & Darling, 2019). Here, negative selection is preferable to positive selection as it avoids any interaction with the surface markers of the desired T cells or APCs, thus by reducing the chance for unwanted unspecific activation of the desired cells. The use of these isolated specific cell types for the LTT platform could reduce the interference or 'background noise' from the other cell types. Moreover, as PBMCs also contain diverse T cell types, including regulatory T cells (Treg) which suppress T cell activation, the removal of such cell types from the PBMC cultures may also likely reduce the suppression of the immune response (Srinoulprasert & Pichler, 2014). Another interesting approach, to reduce the suppression of the immune response may be through the addition of anti-PD-1/anti-PD-L1 (Rosskopf et al., 2018) or anti-CTLA4 antibodies (Hellings et al., 2002) during LTT culturing step, as these antibodies have shown promise in cancer immunotherapy and are also involved in modulating allergic reaction (Seidel, Otsuka, & Kabashima, 2018). On the basis of the T cell type (Th or Tc) or APCs, which is specifically isolated and used in the LTT, it could help in determining the responsive T cell type for the observed drug allergic reaction provided that the *in-vitro* situation reflects the mechanisms taking place *in-vivo*. For instance, Bechara et al. (2019) have used antibody coupled magnetic microbeads to isolate naive Tc and Th cells to characterize Nickel hypersensitivity. Similarly, Antunez et al. (2011) provides evidence for recognition of iodixanol by dendritic cells to increase the cellular response in delayed allergic reactions. In our opinion, the isolation of specific T cell types and APCs using the immunomagnetic beads could easily be integrated prior to the drug incubation step in the classical LTT platform and thus, could increase the sensitivity and specificity of the LTT.

In contrast to an immunomagnetic cell separation approach where a selected cell type is isolated as a population, individual T cells can be counted and analyzed using flow cytometry as a read-out parameter (Martin et al., 2010). Additionally, it is known that various surface markers are upregulated in T cells upon activation such as, CD69 for early activation stage, CD25 for late activation stage, and HLA-DR for even later activation stage (Beeler et al., 2008; Caruso et al., 1997). These markers could be used to characterize the drug specific activated T cells as readout parameter or to isolate them using flow cytometry for downstream analysis. This was demonstrated by Piroird et al. (2015) and Galbiati et al. (2016) where flow cytometry analysis has been employed for measuring the expression of CD86 to assess the activation of dendritic cells upon the chemical sensitization. Furthermore, the importance of flow cytometry for downstream omics analysis is demonstrated in a downstream single cell transcriptomics study, which revealed JAK–STAT signaling pathway as a potential target in a therapy-refractory drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DiHS/DRESS) (Kim et al., 2020). As illustrated by these examples, the quantification of the activated cell type frequency as well as isolation of the activated cells would be possible to monitor cellular changes at a single cell or cell population

[2] The term 'metabolomics or metabolites' refers to the cellular metabolites which constitute normal cellular physiology. It does not refer to drug and/or its *in-vivo* transformed drug products.

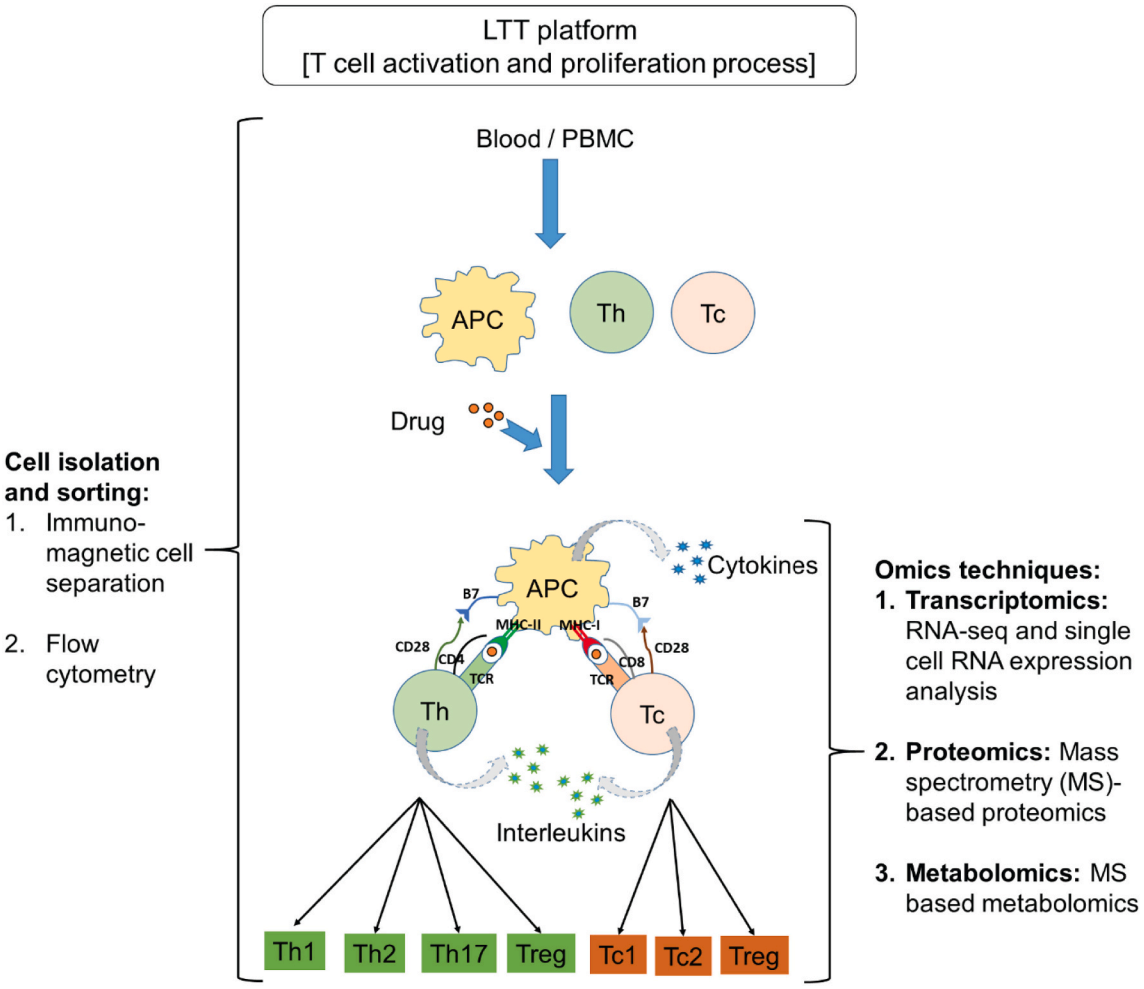

Fig. 1. Implementation of novel techniques at different stages of the T activation and proliferation process in a classical LTT platform. Firstly, immunomagnetic cell separation can be applied prior to the LTT to concentrate the specific immune cells. Later, flow cytometry can be applied after the LTT platform to analyze the individual activated immune cells or to isolate them for the downstream omics analysis. Isolated cells can then be analyzed using transcriptomics, mass spectrometry (MS) based proteomics, and MS based metabolomics techniques to unravel differential molecular changes in drug allergic reactions. [APC: Antigen presenting cells, Th: T helper (CD4+) cells, Tc: cytotoxic T (CD8+) cells, Treg: regulatory T cells]

level. Therefore, we think that flow cytometry, when integrated in the read-out phase of the LTT platform, could be readily used to analyze the activated T cells individually or to further isolate them for the downstream analysis.

Both immunomagnetic cell separation and flow cytometry techniques will be particularly helpful in isolating the specific T cell type prior and after the LTT culturing step, respectively. However, they can also be implemented singly, in tandem or in reverse order in a LTT platform. Both techniques have their limitations and the selection of cell sorting technique depends upon the processing time, cell yields, and viability of resulting cells (Sutermaster & Darling, 2019). In a nutshell, we believe that the approach of immune cells (T cell and/or APCs) isolation by immunomagnetic beads and subsequent co-incubation with drug may increase sensitivity of the LTT. Additionally, flow cytometry analysis of T cells after the LTT will be helpful to quantitatively analyze the specific activated T cells and their corresponding proliferation. This will increase the confidence in the LTT proliferation read-out. Moreover, the collection of immune cells following the aforementioned combined approach will be highly specific to drug allergic reaction and further ease the downstream cellular analysis by various omics techniques.

3. Omics

Modern day omics methods generate quantitative information on an entire complement of expressed genes (transcriptomics), proteins (proteomics), or metabolites (metabolomics). The integration of this information in a systems biology approach is a promising way to enhance our understanding of drug allergy. Here, we discuss these three important omics technologies, what potential they carry if applied in the context of *in-vitro* drug allergy detection, and how they could be implemented in the context of the classical LTT platform (Fig. 1), thereby also furthering our understanding of the molecular changes upon T cell activation and proliferation. Moreover, if they are combined with the cell sorting techniques to focus on selected target cells as described before, the sensitivity and specificity of the downstream cellular analysis could even further be increased.

4. Transcriptomics

As per the central dogma of biology, gene expression directly or indirectly governs all the basic biological processes. Analyzing gene expression is, thus, a powerful and commonly used tool in molecular biology to elucidate basic biological processes, diseases, and specific pathologies. The gene expression analysis, also known as transcriptomics, delivers quantitative information on the expression of single or multiple genes in the cells. It is studied using various techniques ranging from northern blot, reverse transcriptase- real time-PCR (RT-qPCR) for a small number of transcripts, to DNA microarrays, RNA-seq

etc. for multiple transcripts in a high throughput manner (Lennon, 2000; Wang, Gerstein, & Snyder, 2009). These high throughput techniques of global gene expression analysis are now well established and able to deliver detailed information on absolute and relative gene expression levels, gene isoforms, translocation events, and nucleotide variations (Wang et al., 2009). Among the gene expression analysis techniques, RNA-seq by next generation sequencing (NGS) is promising as it can deliver quantitative information on multiple RNA transcripts without prior gene sequence knowledge needed and has also less background noise and greater dynamic range (Wang et al., 2009). Additionally, single cell RNA expression analysis technique which detects RNA molecules directly from the cell extract using fluorescence based complementary probes might also be of interest (Geiss et al., 2008; Tsang et al., 2017), as it can be used to perform relative quantification or analyze gene expression from a single cell (Subkhankulova & Kelsh, 2019). With these respective features, both RNA-seq or single cell RNA expression analysis technique would be well suited for differential gene expression analysis of T cells after a LTT.

The immune response involving drug induced T cell activation and subsequent proliferation is invariably controlled by expression of underlying genes. This is evident in the T cell transcriptomics analysis showing a significant increase in *LAT1 expression, which is involved in* T cell metabolism, and superoxide dismutase 2, interferon-induced transmembrane genes 1 and 2 which are involved in T cell activation and effector functions upon abacavir patch test (Redwood et al., 2019). Upon activation, several co-stimulatory molecules such as ICOS (CD278), 4-1BB (CD137), OX40 (CD134) (Watts, 2005), and diverse cytokines are known to be produced by T cells or APCs (Chen et al., 2018). In line with that, high level of *in-vivo* ICOS expression was observed in Treg cells upon contact hypersensitivity to 2,4-dinitro-fluorobenzene (Vocanson et al., 2010). Similarly, Redwood et al. (2019) showed the significant upregulation of the IFN-γ and IFN-α response pathways in T cells from abacavir-exposed skin. Expression of important stimulatory cytokines such as IL-4, IFN- γ is also significantly upregulated in immediate or delayed type allergic response, respectively (Cornejo-Garcia et al., 2007). Not only mRNA but also certain microRNA (miRNA) populations have altered expression in drug specific Th cells from hypersensitive patients (Monroy-Arreola et al., 2018). Particularly, miR-155 was shown to be up-regulated in PBMCs from all hypersensitive patients 24 h after drug treatment, hinting it to be an early diagnostic biomarker of drug hypersensitivity reactions (Monroy-Arreola et al., 2018). Moreover, various drugs are known to induce drug specific metabolism and corresponding gene expression pattern (Burczynski et al., 2000). These transcription fingerprints could be used to identify the culprit drug in a drug allergic reaction and the mechanism of immune activation by the specific drug type. Similarly, drugs which could inhibit the T cell proliferation could also be identified through their effect on signaling pathways as illustrated in the transcriptomics analysis by Kim et al. (2020) that tofacitinib suppressed T cell proliferation *in-vitro* in DiHS/DRESS through suppressing JAK–STAT signaling pathway (Kim et al., 2020). At the later time points of the LTT, T cell activation culminates into subsequent proliferation through rapid cell divisions (Glinos et al., 2018). The gene expression analysis performed at extended time points should also be able to capture enhanced expression of cell division genes. In overall, transcriptomics analysis using RNA-seq technique for differential gene expression could be able to reveal early and late transcriptional changes in drug allergy and identify associated pathways.

5. Proteomics

The immune response is directly influenced by enzymes and immunological proteins. Therefore, changes in drug allergic reactions are also reflected in the protein composition of the immune cells (Berge et al., 2019). Although gene expression is a precursor to the protein synthesis, it does not necessarily provide the quantitative measure of corresponding protein levels in cells (Gry et al., 2009; Liu, Beyer, & Aebersold, 2016), as post-transcriptional modifications affects the mRNA half-life and translational efficiency. Filling this gap necessitates the qualitative and quantitative proteomics analysis to determine the extent of immune response.

In various fields of science, several methods such as SDS-PAGE, native PAGE, ELISA immunoassays are implemented to analyze few proteins in cells at a time. However, global protein profiling can be possible using high throughput proteomics methods such protein chips, protein microarrays, or mass spectrometry based proteomics (Chandramouli & Qian, 2009). Among them, mass spectrometry based proteomics (MS-proteomics) has been a versatile and commonly used technique for the analysis of a complex protein sample (Chandramouli & Qian, 2009). In MS proteomics, whole proteins or their peptides are ionized and analyzed using mass spectrometer to identify the present proteins. Alongside the protein identification, relative or absolute quantification can be achieved using multiple chemical or metabolic stable isotope labeling methods as reviewed by Lindemann et al. (2017). Moreover, employing the phosphatase inhibitors during the protein extraction procedure, post-translational phosphorylation and dephosphorylation events can also be studied at the protein levels (Phosphoproteomics) (Mayya & Han, 2009).

Early signaling events in T cell activation involves phosphorylation or dephosphorylation at the protein level (Graves & Krebs, 1999; Hwang et al., 2020). T cell antigen receptor (TCR) proximal signaling involves phosphorylation of adapter protein *LAT, through* Lck mediated activation of zeta-chain-associated protein tyrosine kinase *70 (*ZAP-70) (Smith-Garvin, Koretzky, & Jordan, 2009). TCR-induced production of diacylglycerol (DAG) also results in the membrane recruitment of RasGRP, where it is phosphorylated and activated by protein kinase C (PKC) (Smith-Garvin et al., 2009). These and other early signaling events could be captured in a phosphoproteomics analysis of T cells after the short period of drug incubation, as demonstrated by Tan et al. (2017) who identified hundreds of phosphorylation events, mainly among cytoplasmic and cytoskeletal proteins, induced immediately after TCR stimulation. Activated T cells are also known to co-express OX40 (CD134) and IL2RA (CD25) which can be used as a marker for drug-specific activation of CD4+ T cells (Zaunders et al., 2009) as demonstrated in allergic patients who experienced non-immediate reactions to imatinib (Klaewsongkram et al., 2016). Other T cell activation markers such as CD69 (Beeler et al., 2008), PD-1, TNFSF11, and IL-2 (Lochmatter et al., 2009) are also known to be involved in drug allergic response and are gradually upregulated after T cell activation (Tan et al., 2017). The pathway analysis of these proteins shows induction of protein translation, cell metabolism, cell cycle, protein degradation, and cytokine signaling (Tan et al., 2017). As the same proteins and pathways are also likely to be involved in drug-mediated T cell activation, they could bear the potential to be possible read-out parameters for the *in-vitro* detection of drug allergy.

Many cytokines such as IFN- γ and IL-2, IL-5 *etc.* are expressed and released extracellularly during the T cell mediated delayed type drug hypersensitivity (Chen et al., 2018; Lochmatter et al., 2009). Accordingly, their *in-vitro* detection in the LTT platform by ELISA has already been added as an additional read-out parameter to the current LTT platform (Lochmatter et al., 2009; Sachs et al., 2002). Interleukin-15 is also proposed as a marker for early diagnosis and prognosis monitoring as its levels are associated with severity and mortality in Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (Su et al., 2017). Monitoring of cytokines by MS proteomics methods is, however, challenging because of their very low concentrations (Kleiner et al., 2013), which makes it hard to be detected by even sensitive MS methods (Khan, 2012; Mendoza-Porras et al., 2020). Thus, before the LTT supernatant can be analyzed by MS, these low abundant target proteins need to be enriched using immunoaffinity (immunobeads or antibodies) (Bandow, 2010; Millioni et al., 2011). However, care must be taken during the enrichment steps as they can lead to a big loss of low-abundant protein

adhered to the carrier or other non-specific proteins (Ahn & Khan, 2014; Granger et al., 2005). These enrichment steps need to be experimentally tailored to the cytokines analysis in cell media or supernatant (secretome) of the LTT platform. However, if feasible, LTT supernatant analysis will provide the complement of cytokines released during T cell activation, which could widen the spectrum of cytokines as a readout parameter for the LTT. At later time-points of the LTT platform, T cell activation results into T cell proliferation. This will be reflected in enhanced levels of DNA transcription, energy metabolism, and cell division proteins, as shown in integrative proteomics study which identified significant protein enrichment in protein translation (ribosome), cell metabolism, cell cycle, and protein degradation pathways (Tan et al., 2017).

In overall, T cell activation and proliferation will be evident at various levels in phosphoproteome, secretome, and global proteome analysis by MS. However, as many of these changes are very subtle and instantaneous, the appropriate time point selection and snap harvesting of samples for proteomics and phosphoproteomics analysis will likely play a crucial role. Although out of scope of this article, careful sample preparation, sensitive MS data acquisition, and choice of appropriate data analysis workflows is also critical in the proteomics analysis. Moreover, as the LTT platform involves *in-vitro* culturing of PBMCs, MS quantification strategy called 'stable isotope labeling by amino acids in cell culture' (SILAC) (Ong et al., 2002), which involves culturing of target cells in media containing isotope labeled amino acids, might be of interest and amenable in the context of the LTT platform. SILAC uses isotopically labeled amino acids in the growth medium. The cultured T cells in the LTT platform would incorporate these isotopically labeled amino acids into proteins during the T cell proliferation. As MS can distinguish on the basis of mass, multiple samples labeled with different isotopes can be pooled and analyzed together. Thus, SILAC strategy will allow for precise and high confidence relative quantification between control and treatment sample in the LTT platform, as samples can be combined and processed together from the very first step of the experimental MS-proteomics workflow (Chen et al., 2015). The successful implementation of quantitative MS-proteomics in the LTT platform could potentially extend our understanding of the proteins involved in the T cell activation and proliferation process, and identify inducible proteins which could serve as potential read-out parameters or markers for the *in-vitro* detection of drug allergy.

6. Metabolomics

Differential gene and protein expression ultimately affect the intra/extra-cellular metabolites levels, which determines the actual phenotype of cells. Metabolomics delivers identification and quantification of the various metabolites at a given time point. It is performed in targeted mode where only specific metabolites of interest are analyzed, or in an untargeted mode where all detectable metabolites come under revision (Patti, Yanes, & Siuzdak, 2012). Among various analytical techniques, MS based metabolomics is a commonly employed method, owing to its versatility to analyze several chemically different analytes, high sensitivity, and dynamic range (Tsugawa et al., 2014). Here, we explore the scope of MS-metabolomics approach to analyze metabolite changes in the immune cells in the LTT platform.

PBMCs used in a LTT platform are comprised of multiple types of T cells and their effector counterparts (Fig. 1). Different T cells types may have their unique metabolic profiles depending upon the key metabolic regulator (Maclver, Michalek, & Rathmell, 2013; Michalek et al., 2011). Thus, depending upon the kind of drug and the involved key metabolic regulator, different T cells types may respond metabolically very different upon T cell activation and proliferation (Maclver et al., 2013). This forms the basis for our assumption that the metabolic profile of sensitized T cells from allergic individuals will be different from the control T cells (Maclver et al., 2013) and these metabolic differences will be even more amplified upon T cell activation in a positive LTT. The literature on the T cell metabolic changes in drug allergy is sparse but the parallels can be drawn from other metabolic studies involving the T cell activation and proliferation.

It is known that T cell activation results in a rewiring of the cellular metabolism to support consequent rapid growth and cell division. Accordingly, metabolomics studies have already shown that the immune response is associated with strong metabolic changes particularly in the energy metabolism of T cells (Maclver et al., 2013). T cell activation and costimulation promotes the synthesis of the macromolecules to enable the immune response. To fulfil the demand of precursors for macromolecule synthesis and upregulated anabolic pathways, T cells upregulate energy uptake and energy metabolism in terms of glycolysis, glutaminolysis, oxidative phosphorylation, and mitochondrial biogenesis (Chapman, Boothby, & Chi, 2019). Upregulated glucose, glutamine, and arginine utilization by T cells as well as upregulated levels of intermediates from these pathways indicate towards T cell metabolic rewiring. Increased glycolysis in activated T cells is similar to the Warburg effect seen in tumor cells, resulting in pyruvate accumulation or lactate release from the cells (Hiemer et al., 2019). Likewise, increased lactate levels were shown to be directly correlated with the asthma severity in patients (Ostroukhova et al., 2012). von Meyenn, Bertschi, and Schlapbach (2019) has also reviewed recent insights into T cell metabolism and discussed its importance in T cell-mediated skin diseases such as psoriasis and allergic contact dermatitis (von Meyenn et al., 2019). However, these points need to be validated in the wider context of drug allergy.

Not only intracellular metabolism but also nutrient supply in the form of glucose, amino acids and fatty acids, is known to influence the T cell activation and proliferation response (Fox, Hammerman, & Thompson, 2005; Maclver et al., 2013). Particularly, external glucose uptake and utilization plays a critical role in the T cell proliferation response (Fox et al., 2005; Maclver et al., 2013; Michalek et al., 2011). Glycolysis is a required part of the metabolic response of T cells to proliferative signals and T cells do not proliferate in glucose deficient media, even in the presence of alternative energy sources such as the amino acid glutamine (Greiner, Guppy, & Brand, 1994). Therefore, glucose concentration in the LTT media might be an important consideration for the LTT performance. Keeping that in mind, the rapid utilization and resulting depletion of glucose or increase of lactate levels in the media composition of the LTT platform may also form interesting indicators for the T cell proliferation response and thereby the LTT performance.

The IgE mediated immediate (type I) drug allergies exhibit an immediate response through the release of inflammatory mediators (Schnyder & Pichler, 2009). This immediate response results from the crosslinking of drug specific IgE antibodies bound to the FcεRI receptors on the surface of mast cells and basophils, which then release inflammatory mediators such as histamine, heparin, tryptase *etc.* (He et al., 2013; Stone, Prussin, & Metcalfe, 2010). The classical LTT platform, which excludes mast cells (which mainly reside in connective tissues) and basophils (which are circulating in the blood) in the assay, therefore, would not be able to manifest the release of the mentioned inflammatory mediators. However, there are many other *in-vitro* tests available, which focus on the detection of drug specific IgE or subsequent mechanisms but they are subject to separate articles in this "Special Issue on the in vitro detection of drug allergy" (please see: "Basophil and mast cell activation tests by flow cytometry in immediate drug hypersensitivity: diagnosis and beyond" by Elst et al., and "Detection of drug-specific immunoglobulin E (IgE) and measurement of biomarkers for acute drug hypersensitivity reactions" by Brockow et al., 2015).

In case of hapten or pro-hapten drugs, the extent of haptenation may influence the observed immune response. For hapten drugs, the availability of the binding-proteins is important (Cho & Uetrecht, 2017; Sanchez-Gomez et al., 2017; Yvon, Anglade, & Wal, 1990). For pro-hapten drugs, the biotransformation of pro-hapten drugs

(bioactivation) to reactive metabolites, which then bind to proteins to induce the T cell mediated immune response, is important (Chipinda et al., 2011; Cho & Uetrecht, 2017; Naisbitt et al., 2002; Sanderson, Naisbitt, & Kevin Park, 2006). Thus, determining the extent of biotransformation and haptenation is an important but elusive aspect in *in-vitro* assays. In the proposed LTT platform, downstream metabolomics analysis could be used to identify the drug-metabolites levels and thus the extent of drug bioactivation, whereas proteomics could be performed to find out specific drug-protein conjugates (Ariza et al., 2012), which induce the immune response.

Finally, as metabolites are generally the final effector or effected molecules, the metabolites levels in a whole PBMC population or selected T cell types in the final phase of the LTT platform, or released inflammatory mediators may directly correspond to the strength of the allergic immune response. As a vision, we think that the inclusion of metabolic profiling in a LTT platform would not only unravel the induced metabolites and metabolic pathways but also identify metabolite markers for the variable proliferation response observed in a LTT in the context of drug allergy.

7. Conclusion

From the plethora of molecular biology techniques, which could be used to analyze T cell activation in drug allergy, we have illustrated selected techniques, which, from our perspective, are most promising and easily integrable to monitor molecular changes in T cell activation upstream or downstream of the classical LTT platform. The multiplex and high-throughput nature of these techniques could deliver information on several interdependent parameters (cell type counts, expressed genes, proteins, metabolites) which could provide a basis for the discovery of new read-out parameters for the T cell activation in drug allergic reactions *in-vitro*. However, due to the complex nature of immune response in drug allergic reactions, it may not be the single gene or protein or metabolite but rather combinations of thereof which could form the effective panel of markers to detect a drug-specific sensitization *in-vitro*. It is important to reiterate at this point that these molecular changes are dependent upon the state of the T cell in an immune cascade and thus are very rapid, transitory and time-bound. Thus, choosing the right time point for the sample collection is of paramount importance. Additionally, due to low amount of cells or sample in terms of RNA, proteins or metabolites and associated inherent biological variability, micro-sample preparation methods and experimental consistency will be of extremely importance while dealing with these samples. With appropriate time-point selection and processing, these approaches could be able to unravel the key molecular changes and complement the current LTT platform with new read-out parameters for the *in-vitro* detection of drug-specific T cell sensitization, which in combination with other diagnostic findings allows for the conclusion of a drug allergy.

Acknowledgement

We thank the European fund for regional development (EFRE), the German Federal State North Rhine-Westphalia (LeitmarktAgentur. NRW), Federal Institute for Drugs and Medical Devices (BfArM), and the Leibniz Institute for Analytical Sciences - ISAS-e.V. for the research project grant. The position of A.F. is financed by the research grant.

Funding source

The manuscript was written in context with a study related to the improvement of the lymphocyte transformation test which was funded by the European Fund for Regional Development (EFRE) and the German Federal State North Rhine-Westphalia (LeitmarktAgentur. NRW) (funding number: EFRE-0801755) and from own resources of the Federal Institute for Drugs and Medical Devices (BfArM) and the Leibniz Institute for Analytical Sciences - ISAS-e.V., Dortmund, Germany.

References

Ahn, Seong-Beom, Khan, Alamgir, 2014. Detection and quantitation of twenty-seven cytokines, chemokines and growth factors pre-and post-high abundance protein depletion in human plasma. EuPA Open Proteomics 3, 78–84.
Antunez, C., et al., 2011. Recognition of iodixanol by dendritic cells increases the cellular response in delayed allergic reactions to contrast media. Clinical & Experimental Allergy 41 (5), 657–664.
Ariza, Adriana, et al., 2012. Protein haptenation by amoxicillin: High resolution mass spectrometry analysis and identification of target proteins in serum. Journal of Proteomics 77, 504–520.
Bandow, Julia E., 2010. Comparison of protein enrichment strategies for proteome analysis of plasma. Proteomics 10 (7), 1416–1425.
Bechara, Rami, et al., 2019. Identification and characterization of circulating naïve CD4 + and CD8+ T cells recognizing nickel. Frontiers in Immunology 10, 1331.
Beeler, Andreas, et al., 2006. Long-lasting reactivity and high frequency of drug-specific T cells after severe systemic drug allergy reactions. Journal of Allergy and Clinical Immunology 117 (2), 455–462.
Beeler, Andreas, et al., 2008. CD69 upregulation on T cells as an *in-vitro* marker for delayed-type drug allergy. Allergy 63 (2), 181–188.
Berge, Tone, et al., 2019. Quantitative proteomic analyses of CD4+ and CD8+ T cells reveal differentially expressed proteins in multiple sclerosis patients and healthy controls. Clinical Proteomics 16 (1), 19.
Brockow, K., et al., 2015. Guideline for the diagnosis of drug hypersensitivity reactions: S2K-Guideline of the German Society for Allergology and Clinical Immunology (DGAKI) and the German Dermatological Society (DDG) in collaboration with the Association of German Allergologists (AeDA), the German Society for Pediatric Allergology and Environmental Medicine (GPA), the German Contact Dermatitis Research Group (DKG), the Swiss Society for Allergy and Immunology (SGAI), the Austrian Society for Allergology and Immunology (OGAI), the German Academy of Allergology and Environmental Medicine (DAAU), the German Center for Documentation of Severe Skin Reactions and the German Federal Institute for Drugs and Medical Products (BfArM). Allergo Journal of International 24 (3), 94–105.
Burczynski, Michael E., et al., 2000. Toxicogenomics-based discrimination of toxic mechanism in HepG2 human hepatoma cells. Toxicological sciences 58 (2), 399–415.
Caruso, Arnoldo, et al., 1997. Flow cytometric analysis of activation markers on stimulated T cells and their correlation with cell proliferation. Cytometry: The Journal of the International Society for Analytical Cytology 27 (1), 71–76.
Chandramouli, Kondethimmanahalli, Qian, Pei-Yuan, 2009. Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Human Genomics and Proteomics: HGP 2009.
Chapman, Nicole M., Boothby, Mark R., Chi, Hongbo, 2019. Metabolic coordination of T cell quiescence and activation. Nature Reviews Immunology 1–16.
Chen, Chun-Bing, et al., 2018. An updated review of the molecular mechanisms in drug hypersensitivity. Journal of Immunology Research 2018.
Chen, Xiulan, et al., 2015. Quantitative proteomics using SILAC: Principles, applications, and developments. Proteomics 15 (18), 3175–3192.
Chipinda, Itai, Hettick, Justin M., Siegel, Paul D., 2011. Haptenation: chemical reactivity and protein binding. Journal of Allergy 2011.
Cho, Tiffany, Uetrecht, Jack, 2017. How reactive metabolites induce an immune response that sometimes leads to an idiosyncratic drug reaction. Chemical research in toxicology 30 (1), 295–314.
Cornejo-Garcia, J.A., et al., 2007. Differential cytokine and transcription factor expression in patients with allergic reactions to drugs. Allergy 62 (12), 1429–1438.
Fox, Casey J., Hammerman, Peter S., Thompson, Craig B., 2005. Fuel feeds function: energy metabolism and the T-cell response. Nature Reviews Immunology 5 (11), 844–852.
Galbiati, Valentina, et al. "In vitro models to evaluate drug-induced hypersensitivity: potential test based on activation of dendritic cells." Frontiers in Pharmacology 7 (2016): 204.
Geiss, Gary K., et al., 2008. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nature biotechnology 26 (3), 317–325.
Glinos, Dafni A., et al., 2018. Genomic profiling of T cell activation reveals dependency of memory T cells on CD28 costimulation. BioRxiv 421099.
Granger, Jill, et al., 2005. Albumin depletion of human plasma also removes low abundance proteins including the cytokines. Proteomics 5 (18), 4713–4718.
Graves, Jonathan D., Krebs, Edwin G., 1999. Protein phosphorylation and signal transduction. Pharmacology & therapeutics 82 (2–3), 111–121.
Greiner, E.F., Guppy, M., Brand, K., 1994. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. Journal of Biological Chemistry 269, 31484–31490.
Gry, Marcus, et al., 2009. Correlations between RNA and protein expression profiles in 23 human cell lines. BMC genomics 10 (1), 1–14.
He, Shao-heng, et al., 2013. Mast cells and basophils are essential for allergies: mechanisms of allergic inflammation and a proposed procedure for diagnosis. Acta Pharmacologica Sinica 34 (10), 1270–1283.
Hellings, Peter W., et al., 2002. Blockade of CTLA-4 enhances allergic sensitization and eosinophilic airway inflammation in genetically predisposed mice. European journal of immunology 32 (2), 585–594.
Hiemer, Samantha, et al., 2019. Integrated metabolomic and transcriptomic profjiling reveals novel activation-induced metabolic networks in human T cells. BioRxiv 635789.

Horgan, Kevin, Shaw, Stephen, Boirivant, Monica, 2009. Immunomagnetic purification of T cell subpopulations. Current Protocols in Immunology 85 (1), 4–7.
Huber, H., Huber, C., Braunsteiner, H., 1966. The clinical significance of lymphocyte culture. In-vitro transformation as a test of lymphocyte function. German Medical Monthly 11 (7), 289.
Hwang, Jeong-Ryul, et al., 2020. Recent insights of T cell receptor-mediated signaling pathways for T cell activation and development. Experimental & Molecular Medicine 1–12.
Ikeda, Takaaki, et al., 1998. Flow cytometric method to detect lymphocyte transformation in drug-allergic hepatic injury. Digestive diseases and sciences 43 (3), 513–520.
Kalish, Richard S., et al., 1994. Sulfonamide-reactive lymphocytes detected at very low frequency in the peripheral blood of patients with drug-induced eruptions. Journal of allergy and clinical immunology 94 (3), 465–472.
Karami, Zahra, et al., 2016. Evaluation of lymphocyte transformation test results in patients with delayed hypersensitivity reactions following the use of anticonvulsant drugs. International Archives of Allergy and Immunology 170 (3), 158–162.
Khan, Alamgir, 2012. Detection and quantitation of forty eight cytokines, chemokines, growth factors and nine acute phase proteins in healthy human plasma, saliva and urine. Journal of Proteomics 75 (15), 4802–4819.
Kim, Doyoung, et al., 2020. Targeted therapy guided by single-cell transcriptomic analysis in drug-induced hypersensitivity syndrome: a case report. Nature medicine 26 (2), 236–243.
Klaewsongkram, Jettanong, et al., 2016. Slow desensitization of imatinib-induced nonimmediate reactions and dynamic changes of drug-specific CD4+ CD25+ CD134 + lymphocytes. Annals of Allergy, Asthma & Immunology 117 (5), 514–519.
Kleiner, Giulio, et al., 2013. Cytokine levels in the serum of healthy subjects. Mediators of inflammation 2013.
Lennon, Gregory G., 2000. High-throughput gene expression analysis for drug discovery. Drug Discovery Today 5 (2), 59–66.
Levine, Bernard B., Ovary, Zoltan, 1961. Studies on the mechanism of the formation of the penicillin antigen: III. The N-(d-α-benzylpenicilloyl) group as an antigenic determinant responsible for hypersensitivity to penicillin G. The Journal of experimental medicine 114 (6), 875–940.
Lindemann, Claudia, et al., 2017. Strategies in relative and absolute quantitative mass spectrometry based proteomics. Biological chemistry 398 (5–6), 687–699.
Liu, Yansheng, Beyer, Andreas, Aebersold, Ruedi, 2016. On the dependency of cellular protein levels on mRNA abundance. Cell 165 (3), 535–550.
Lochmatter, P., et al., 2009. Drug-specific in vitro release of IL-2, IL-5, IL-13 and IFN-γ in patients with delayed-type drug hypersensitivity. Allergy 64 (9), 1269–1278.
Lopez, S., et al., 2009. Lymphocyte proliferation response in patients with delayed hypersensitivity reactions to heparins. British Journal of Dermatology 160 (2), 259–265.
Luque, I., et al., 2008. In vitro T-cell responses to β-lactam drugs in immediate and nonimmediate allergic reactions. Allergy 56 (7), 611–618.
Maclver, Nancie J., Michalek, Ryan D., Rathmell, Jeffrey C., 2013. Metabolic regulation of T lymphocytes. Annual review of immunology 31, 259–283.
Martin, M., et al., 2010. In vitro detection and characterization of drug allergy using flow cytometry. Allergy 65 (1), 32–39.
Mayorga, Cristobalina, et al., 2017. The value of *in-vitro* tests to diminish drug challenges. International Journal of Molecular Sciences 18 (6), 1222.
Mayya, Viveka, Han, David K., 2009. Phosphoproteomics by mass spectrometry: Insights, implications, applications and limitations. Expert Review of Proteomics 6 (6), 605–618.
Mendoza-Porras, Omar, et al., 2020. Cytokines in the grass, a lesson learnt: Measuring cytokines in plasma using multiple reaction monitoring mass spectrometry. Rapid Communications in Mass Spectrometry 34 (9), e8723.
Michalek, Ryan D., et al., 2011. Cutting edge: Distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. The Journal of Immunology 186 (6), 3299–3303.
Millioni, Renato, et al., 2011. High abundance proteins depletion vs low abundance proteins enrichment: Comparison of methods to reduce the plasma proteome complexity. PLoS One 6 (5), e19603.
Monroy-Arreola, Alejandra, et al., 2018. Up-regulation of T-cell activation microRNAs in drug-specific CD4+ T-cells from hypersensitive patients. Chemical Research in Toxicology 31 (6), 454–461.
Naisbitt, Dean J., et al., 2002. Covalent binding of the nitroso metabolite of sulfamethoxazole leads to toxicity and major histocompatibility complex-restricted antigen presentation. Molecular pharmacology 62 (3), 628–637.
Ong, Shao-En, et al., 2002. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Molecular & Cellular Proteomics 1 (5), 376–386.
Ostroukhova, M., Goplen, N., Karim, M.Z., Michalec, L., Guo, L., et al., 2012. The role of low-level lactate production in airway inflammation in asthma. American Journal of Physiology Lung Cellular Molecular Physiology 302, L300–L307.
Patti, Gary J., Yanes, Oscar, Siuzdak, Gary, 2012. Metabolomics: The apogee of the omics trilogy. Nature reviews Molecular cell biology 13 (4), 263–269.
Pichler, W.J., Tilch, J., 2004. The lymphocyte transformation test in the diagnosis of drug allergy. Allergy 59 (8), 809–820.
Pichler, Werner J., 2019. Immune pathomechanism and classification of drug hypersensitivity. Allergy 74 (8), 1457–1471.
Piroird, Cécile, et al., 2015. The Myeloid U937 Skin Sensitization Test (U-SENS) addresses the activation of dendritic cell event in the adverse outcome pathway for skin sensitization. Toxicology in vitro 29 (5), 901–916.
Porebski, G., et al., 2013. In vitro drug causality assessment in Stevens–Johnson syndrome–alternatives for lymphocyte transformation test. Clinical & Experimental Allergy 43 (9), 1027–1037.
Redwood, Alec James, et al., 2019. Single-cell transcriptomics reveal polyclonal memory T cell responses in skin with positive abacavir patch test results. Journal of Allergy and Clinical Immunology 144 (5), 1413–1416.
Rosskopf, Sandra, et al., 2018. PD-1 has a unique capacity to inhibit allergen-specific human CD4+ T cell responses. Scientific reports 8 (1), 1–11.
Sachs, B., et al., 2001. In vitro drug allergy detection system incorporating human liver microsomes in chlorazepate-induced skin rash: drug-specific proliferation associated with interleukin-5 secretion. British Journal of Dermatology 144 (2), 316–320.
Sachs, B., et al., 2002. Determination of interleukin-5 secretion from drug-specific activated ex vivo peripheral blood mononuclear cells as a test system for the *in-vitro* detection of drug sensitization. Clinical & Experimental Allergy 32 (5), 736–744.
Sanchez-Gomez, Francisco J., et al., 2017. Amoxicillin haptenates intracellular proteins that can be transported in exosomes to target cells. Allergy 72 (3), 385–396.
Sanderson, Joseph P., Naisbitt, Dean J., Kevin Park, B., 2006. Role of bioactivation in drug-induced hypersensitivity reactions. The AAPS Journal 8 (1), E55–E64.
Sarkany, I., 1967. Lymphocyte transformation in drug allergy. The Lancet 289 (7493), 743–745.
Schnyder, Benno, Pichler, Werner J., 2009. Mechanisms of drug-induced allergy. In: Mayo clinic proceedings, Vol. 84. Elsevier. No. 3.
Seidel, Judith A., Otsuka, Atsushi, Kabashima, Kenji, 2018. Anti-PD-1 and anti-CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations. Frontiers in oncology 8, 86.
Smith-Garvin, Jennifer E., Koretzky, Gary A., Jordan, Martha S., 2009. T cell activation. Annual review of immunology 27, 591–619.
Srinoulprasert, Yuttana, Pichler, Werner J., 2014. Enhancement of drug-specific lymphocyte proliferation using CD25hi-depleted CD3+ effector cells. International Archives of Allergy and Immunology 163 (3), 198–205.
Stone, Kelly D., Prussin, Calman, Metcalfe, Dean D., 2010. IgE, mast cells, basophils, and eosinophils. Journal of Allergy and Clinical Immunology 125 (2), S73–S80.
Su, Shih-Chi, et al., 2017. Interleukin-15 is associated with severity and mortality in Stevens-Johnson syndrome/toxic epidermal necrolysis. Journal of Investigative Dermatology 137 (5), 1065–1073.
Subkhankulova, Tatiana, Kelsh, Robert Neil, 2019. Fluorescence-activated cell sorting and nanostring profiling of single neural crest cells and pigment cells. In: Neural crest cells. Humana Press, New York, NY, pp. 185–193.
Sutermaster, Bryan A., Darling, Eric M., 2019. Considerations for high-yield, high-throughput cell enrichment: Fluorescence versus magnetic sorting. Scientific Reports 9 (1), 1–9.
Suthumchai, N., et al., 2018. The measurement of drug-induced interferon γ-releasing cells and lymphocyte proliferation in severe cutaneous adverse reactions. Journal of the European Academy of Dermatology and Venereology 32 (6), 992–998.
Tan, Haiyan, et al., 2017. Integrative proteomics and phosphoproteomics profiling reveals dynamic signaling networks and bioenergetics pathways underlying T cell activation. Immunity 46 (3), 488–503.
Trautmann, A., et al., 2014. Aminopenicillin-associated exanthem: Lymphocyte transformation testing revisited. Clinical & Experimental Allergy 44 (12), 1531–1538.
Tsang, Hin-Fung, et al., 2017. Nanostring, a novel digital color-coded barcode technology: Current and future applications in molecular diagnostics. Expert review of molecular diagnostics 17 (1), 95–103.
Tsugawa, Hiroshi, et al., 2014. Highly sensitive and selective analysis of widely targeted metabolomics using gas chromatography/triple-quadrupole mass spectrometry. Journal of Bioscience and Bioengineering 117 (1), 122–128.
Vocanson et al, 2010; Inducible costimulator (ICOS) is a marker for highly suppressive antigen-specific T cells sharing features of TH17/TH1 and regulatory T cells.
von Meyenn, Leonhard, Bertschi, Nicole Leonie, Schlapbach, Christoph, 2019. Targeting T cell metabolism in inflammatory skin disease. Frontiers in Immunology 10, 2285.
Wang, Zhong, Gerstein, Mark, Snyder, Michael, 2009. RNA-Seq: A revolutionary tool for transcriptomics. Nature reviews genetics 10 (1), 57–63.
Watts, Tania H., 2005. TNF/TNFR family members in costimulation of T cell responses. Annual Review of Immunology 23, 23–68.
Weltzien, Hans Ulrich, et al., 1996. T cell immune responses to haptens. Structural models for allergic and autoimmune reactions. Toxicology 107 (2), 141–151.
Yun, James, et al., 2016. T-cell-mediated drug hypersensitivity: immune mechanisms and their clinical relevance. Asia Pacific Allergy 6 (2), 77.
Yvon, Mireille, Anglade, Patricia, Wal, Jean-Michel, 1990. Identification of the binding sites of benzyl penicilloyl, the allergenic metabolite of penicillin, on the serum albumin molecule. FEBS letters 263 (2), 237–240.
Zaunders, John J., et al., 2009. High levels of human antigen-specific CD4+ T cells in peripheral blood revealed by stimulated coexpression of CD25 and CD134 (OX40). The Journal of Immunology 183 (4), 2827–2836.