Drug hypersensitivity and eosinophilia: The decisive role of p-i stimulation

Werner J. Pichler | Lester Thoo | Daniel Yerly

ADR-AC GmbH, Bern, Switzerland

Correspondence
Werner J. Pichler, ADR-AC GmbH, Holligenstrasse 91, 3008 Bern, Switzerland.
Email: werner.pichler@adr-ac.ch

Abstract
Eosinophilia is a common finding in drug hypersensitivity reactions (DHR). Its cause is unclear, as neither antigen/allergen-driven inflammation nor clonal expansion is involved. Most delayed-DHRs are due to p-i (pharmacologic interaction of drugs with immune receptors). These are off-target activities of drugs with immune receptors that result in various types of T-cell stimulation, some of which involve excessive IL-5 production. Functional and phenotypic studies of T-cell clones and their TCR-transfected hybridoma cell lines revealed that some p-i-induced drug stimulations occur without CD4/CD8 co-receptor engagement. The CD4/CD8 co-receptors link Lck (lymphocyte-specific protein tyrosine kinase) and LAT (linker for activation of T cells) to the TCR. Alteration of Lck or LAT can result in a TCR signalosome with enhanced IL-5 production. Thus, if a more affine TCR-[drug/peptide/HLA] interaction allows bypassing the CD4 co-receptor, a modified Lck/LAT activation may lead to a TCR signalosome with elevated IL-5 production. This "IL-5-TCR-signalosome" hypothesis could also explain eosinophilia in superantigen or allo-stimulation (graft-versus-host disease), in which evasion of CD4/CD8 co-receptors has also been described. It may open new therapeutic possibilities in certain eosinophilic diseases by directly targeting the IL-5-TCR signalosome.

KEYWORDS
CD4/CD8 co-receptors, drug hypersensitivity, eosinophilia, IL-5, p-i concept, TCR signalosome

1 | INTRODUCTION

Drug hypersensitivity reactions (DHRs) are immune and/or inflammatory responses triggered by a drug that become clinically evident. Often, the drug is a relatively small molecule (<1000D). Some peptides/proteins also serve as drugs. However, the immune responses they elicit follow different rules and are not discussed here.

Initially, DHRs were explained by the hapten–protein concept, in which a small chemical, drug or drug metabolite, called hapten, binds covalently to a protein. This forms a new hapten–protein complex that acts as a new antigen, triggering a specific immune response involving T cells and antibodies. This hapten/antigen mechanism is well established in contact dermatitis.

More relevant in systemic drug reactions is the so-called p-i concept (pharmacologic interaction of drugs with immune receptors).
It states that drugs (or a metabolite, e.g., oxypurinol) stimulate the immune system through direct, non-covalent binding to immune receptors themselves.\(^1\)\(^-\)\(^7\) This process begins with the drug’s off-target activity on TCR or HLA, accumulating non-covalent drug-protein interactions until specific avidity is reached. The clinically relevant signal always originates from activated T cells when the drug binds to TCR/T cell itself or to HLA on antigen-presenting cells or tissue cells.\(^5\)\(^-\)\(^7\) Interestingly, this p-i stimulation often results in a surprisingly strong immune stimulation—even though the drug does not act as an antigen!

p-i stimulations are similar to superantigen or allo-antigen stimulations.\(^5\)\(^-\)\(^8\) In particular, the high cytotoxicity observed in almost all delayed DHRs has been associated with allo-like stimulations.\(^5\)\(^-\)\(^8\) For instance, in abacavir-induced DHR, around 5% of abacavir-induced CD8\(^+\) T cells react with the allo-allele B*58:01, which is structurally similar to (B*57:01 + abacavir).\(^8\)

Here, we address eosinophilia, another prominent characteristic of delayed-onset DHRs. It is present in approximately 50% of DHRs and is a defining characteristic of some severe reactions, such as DRESS (drug reaction with eosinophilia and systemic symptoms).\(^9\)\(^-\)\(^11\)

Eosinophilia in drug hypersensitivity (EOS-DH) is different from eosinophilia caused by allergic/atopic inflammation or clonal expansion due to rearrangements in certain oncogenic target genes.\(^12\)\(^-\)\(^13\) We hypothesize that EOS-DH arises from an “aberrant” TCR signalosome due to p-i stimulation, leading to excessive IL-5 production (termed “IL-5 TCR signalosome”). This hypothesis is supported by the distinct attributes of p-i stimulated, drug-specific T-cell clones (TCC), that produce high levels of IL-5, as well as the understanding of CD4/CD8 linked Lck and LAT proteins in the TCR signalosome, particularly their ability to increase the production of Th2 cytokines.\(^16\)\(^-\)\(^17\)

2 | HETEROGENEITY AND PECULIARITY OF p-i

The p-i concept summarizes many different drug-immune receptor interactions (Figure 1).\(^5\)\(^-\)\(^6\)\(^,\)\(^18\) To distinguish between these “p-i stimulations,” a straightforward approach is to categorize them as either p-i HLA or p-i TCR, depending on which immune receptor the drug primarily binds to.\(^5\)\(^-\)\(^6\)\(^,\)\(^18\) Nonetheless, there are subtle yet functionally relevant variations within p-i HLA or p-i TCR.\(^19\)\(^-\)\(^22\) For instance, in abacavir hypersensitivity, when the drug binds to HLA within the cell (endoplasmatic reticulum), it can trigger a modified peptide presentation (Figure 1F).\(^23\)\(^-\)\(^25\) Binding of sulfamethoxazole (SMX) to TCR-CDR2\(^\beta\) can elicit an allosteric effect on the TCR, leading to increased affinity for peptide antigens (Figure 1C).\(^19\) Moreover, drug binding to a peptide loop within TCR-CDR3\(\alpha\) could be stimulating, irrespective of the type of interacting peptide/HLA or the involvement of co-receptor CD4/CD8 (Figure 1B).\(^21\)\(^-\)\(^26\)\(^,\)\(^27\)

Thus, different drug-immune receptor interactions lead to different T-cell signals. Notably, these various p-i stimulations often occur together. For example, SMX can stimulate via binding to TCR-CDR3\(\alpha\) (Figure 1B),\(^20\) via allosteric action on TCR-CDR2\(\beta\) (Figure 1C),\(^19\) by binding to HLA (Figure 1D),\(^28\) and even as a hapten (SMX-NO\(^2\))\(^29\)\(^,\)\(^30\) (Figure 1A) in the same individual. This heterogeneity of drug/p-i stimulations may explain the complexity of clinical DH symptoms within one individual: some p-i stimulations result in expansion of TCC which produce high amounts of IL-5, while other TCC are characterized by cytotoxicity. Thereby, depending on the genetic background, a specific p-i stimulation may become clinically dominant: for example, carbamazepine tends to elicit DRESS in HLA A*31:01, but Stevens–Johnson syndrome (SJS) in B*15:02 carriers.\(^28\)\(^,\)\(^31\)

The different p-i stimulations result in a heterogeneous phenotype/reaction pattern of drug-responsive T cells with often unusual characteristics (Figure 2).\(^32\)\(^-\)\(^35\) Of particular, relevance and potential relation to the induction of EOS-DH may be the observation that CD4/CD8 co-receptor engagements can be omitted during drug-induced T-cell stimulations: CD4\(^+\) TCC react with HLA class I and CD8\(^+\) TCC with HLA II.\(^26\)\(^,\)\(^27\) In addition, many drug-induced TCCs are double positive (CD4\(^+\)/CD8\(^+\)).\(^34\)\(^-\)\(^39\) Transfection of drug-specific TCR into hybridoma cell lines lacking human CD4/CD8 co-receptors receptors have certain limitations (e.g., a restricted evaluation of cell stimulations), but revealed solid and dose-dependent reactions.\(^40\)\(^,\)\(^41\)

These data suggest that some drug-TCR interactions are more affine than others and that T-cell activation does not necessitate co-receptor engagement. Figure 2 summarizes these and other peculiar findings of p-i stimulations (Figure 2).\(^5\)\(^-\)\(^8\)\(^,\)\(^32\)\(^-\)\(^42\)

3 | EOSINOPHILIA DUE TO p-i STIMULATION

A prominent characteristic of delayed-appearing DHR is eosinophilia. Blood eosinophilia is defined by an absolute eosinophil count of more than 0.5 × 10\(^3\)/L, whereas hypereosinophilia requires a total eosinophil count of ≥1.5 × 10\(^3\)/L.\(^12\)\(^,\)\(^13\)\(^,\)\(^43\)

Eosinophilia is observed in approximately 80% of patients with DRESS, sometimes even with eosinophil counts of over 1.5 × 10\(^3\)/L.\(^11\) Around half of patients with MPE exhibit eosinophilia.\(^9\)\(^,\)\(^16\) Some patients with AGEP may also develop an eosinophilia in addition to neutrophilia.\(^44\) Elevated serum levels of IL-5, the cytokine that promotes eosinophil differentiation, maturation, survival, activation, and migration can be detected in these DH patients.\(^9\)\(^,\)\(^45\)\(^-\)\(^47\)

In vitro stimulation of PBMC obtained from patients with DHR (MPE and particularly DRESS) revealed that patients’ T cells secreted high levels of IL-5 upon drug stimulation.\(^34\)\(^,\)\(^15\) These levels often exceed the amount of IL-5 induced by a mitogen like pokeweed mitogen (PWM) (personal observation of cytotoxic values obtained in a 6-day culture of patients’ cells with the culprit drug). Cloning these drug-stimulated T cells revealed that some drug-specific TCCs secreted very high amounts of IL-5 (>400 μg/mL IL-5 per 10\(^6\) cells/48h of lidocaine-specific TCC). However, the individual TCCs varied substantially in cytokine secretion and cytotoxicity.\(^14\)\(^,\)\(^1,\)\(^37\)\(^-\)\(^39\)
Mechanism of eosinophilia

Eosinophilia is normally linked to two fundamentally different mechanisms (Table 1). Clonal expansion:

Rearrangements in certain oncogenic target genes, such as PDGFRA, PDGFRB, fibroblast growth factor receptor-1 (FGFR1), JAK2, ABL1, and ETV6, can lead to the accumulation of clonal eosinophils in the skin, blood, and other organs resulting in hypereosinophilic syndrome (HES). The lymphocytic variant HES (L-HES) is a particular form of clonal expansion in which T cells with an aberrant immunophenotype produce excessive amounts of IL-5 and cause tissue eosinophilia with varying degrees of epidermal involvement and eosinophilic infiltrates.

Reactive forms:

Reactive eosinophilia, caused by an antigen/allergen-driven process, is far more common than the neoplastic forms. When an antigen/allergen enters the body through the skin, respiratory or gastrointestinal tract, it encounters epithelial cells and innate lymphoid cells (ILC). This leads to eosinophilic inflammation due to cytokine-mediated (IL-3, IL-5, GM-CSF) differentiation and survival of eosinophils. The most common manifestation of such eosinophilic diseases are atopic dermatitis, asthma, and allergic rhinosinusitis. It is likely that many parasitic worm infections also belong to this reactive subgroup of eosinophilia since helminth infection is typically associated with a strong Th2 immune response.
response, and eosinophils can effectively kill or damage larvae and adult worms in vitro (Table 1).

3.2 | Eosinophilia in DH (EOS-DH)

Understanding the sequence of events leading to EOS-DH is crucial to distinguishing it from reactive, antigen-driven forms of eosinophilia. Drugs distribute rapidly in the tissue and bind to proteins or receptors. DH starts without prior tissue priming by an allergen/antigen. It can occur when a drug shows a functionally relevant off-target activity with immune receptors, resulting in immediate cell activation. Since only a few cells can initially react, no clinical symptoms occur. Under continuous drug therapy (most delayed T-cell reactions occur after days or weeks of therapy), the lymphocytes are further activated, expand and migrate into the target tissue (usually skin, sometimes liver, etc.). This development of a DHR until the appearance of symptoms takes time and is therefore delayed.
The p-i activated lymphocytes are often cytotoxic, and an early and common sign of DHR in tissue may be hydropic degeneration caused by infiltrating perforin-/-granzyme- cytotoxic T cells. In addition, p-i stimulation leads to the secretion of Th1 and (often) Th2-related cytokines and chemokines. For example, IFNγ may activate tissue cells and enhance LFA1 and HLA-class II expression.

When does eosinophilia accompany DHR? Eosinophilia may be an additional, but not mandatory, component that occurs in various p-i-induced T-cell stimulations. It may occur when some drug-induced p-i stimulations can form an IL-5 TCR signalosome. IL-5 (and IL-13, which is also frequently produced) then acts in synergy with the chemoattractants eotaxin-1 (CCL11) and thymus- and activation-regulated chemokine (TARC/CCL17). They are probably locally induced by the T-cell-derived cytokines IFNγ and TNFα. This sequence of events (T cell first, then tissue activation) contrasts with a coordinated, antigen/allergen-driven immune response in which Th0 to Th2 polarization develops under the influence of cytokines and marks the end of a cascade of events.

The level of eosinophilia may depend on the amount of IL-5-secreting T cells, which appears to be high in DRESS, lower in MPE and even absent in others DHRs. The symptoms of DHR including the eosinophilic reaction are transient, similar to the reactive, antigen-driven form, and they typically disappear after discontinuation of drug therapy.

### 3.3 | An “aberrant” TCR signalosome may lead to increased IL-5 production and eosinophilia

The activation of T cells is an organized and complex process that depends on TCR signaling. It requires an orchestrated interplay of receptors, receptor-associated factors, kinases, and transcription factors, collectively known as the “TCR signalosome” (shown in Figure 3).

Two proteins associated with CD4/CD8 co-receptor engagement of the TCR-signalosome (Figure 3) may play a crucial role in DH/p-i-related eosinophilia. The first is the lymphocyte-specific protein tyrosine kinase (Lck), an Src kinase family member. It is critical for transducing cell membrane signals that activate intracellular signaling cascades. Lck has been reported to be associated with the co-receptors CD4 and CD8 in T cells. However, TCR signaling can also occur in the absence of CD4 or CD8 co-receptor involvement, likely by engaging membrane-bound Lck, not linked to CD4/CD8. T cells that lack Lck produce dysregulated levels of Th2-type cytokines (Figure 3).

Another important protein in the TCR signalosome associated with CD4/CD8 co-receptors is LAT (linker for activation of T cells). LAT is an adaptor protein whose tyrosine phosphorylation is critical for transmitting the TCR signal. In common TCR-peptide-HLA interactions, CD4 or CD8 engagement brings LAT closer to protein tyrosine kinase ZAP 70, which phosphorylates LAT. The resulting “LAT signalosome” links the TCR to primary intracellular signaling pathways that regulate T-cell development and function. Malissen et al. have identified mutations in LAT that can lead to excessive Th2 cytokine production (IL-4, IL-13, IL-5) with massive eosinophilia in mice. A family with a certain LAT mutation, immunodeficiency, and a Th2 bias has been described by Keller et al. Thus, it appears that the LAT protein is also involved in promoting IL-5 (Th2 cytokines). One possible explanation for the effect of certain p-i stimulations on the T-cell function is that a delayed/reduced phosphorylation of LAT by ZAP70 occurs when membrane-LCK instead of CD4-linked LCK is involved in the signalosome (Figure 3B).

The role of CD4 and CD8 co-receptors, which bring Lck and LAT closer to the TCR signalosome, differs in CD4 or CD8: the role of CD8–LCK in peripheral T cells seems to be largely limited to enhancing the signaling induced by low-affinity antigens. In contrast, CD4-bound LCK is required for efficient development and function of helper T cells via kinase-independent stabilization of surface CD4. Thus, bypassing of CD4 co-receptor upon p-i stimulation and signaling via membrane-bound or free LCK may be the relevant signal for the induction of IL-5 in CD4 cells, as the phosphorylation of TCR-complex differs between free and co-receptor-bound LCK. The hypothetical effects of bypassing CD4/CD8 co-receptors on Lck/LAT activation and on TCR signalosome on IL-5 production are shown in Figure 3B.

To illustrate the role of CD4/CD8 co-receptors on the TCR signalosome and IL-5 production, CD4–HLA I or CD8–HLA II mismatched TCC serve as an example for an “aberrant” TCR signalosome. In such TCC, the HLA I/II-mismatched CD4/CD8 co-receptor binding might not occur. However, it is possible that an “aberrant” TCR signalosome may also occur in CD4/CD8–HLA-matched p-i stimulations. Then, the CD4 (or CD8) co-receptor may be present but is not or only partially used, for example, if the drug-TCR interaction per se is sufficiently affine and stable. Despite their availability, the co-receptors would not link Lck/LAT to the signalosome in the usual manner. Thus, high IL5 eosinophilia might occur even with matched (CD4–HLA II) TCR-APC combinations, if the affinity for the ligand (HLA-peptide or drug) is high.

Only a portion of the TCCs produced found were HLA–CD4/CD8–mismatched or double positive. However, because IL-5 production per single cell can be very high, it may be sufficient to cause eosinophilia. The majority of drug-induced T cells appear to be stimulated by other p-i stimulations (Figure 1), which likely have low affinity for the drug/peptide/HLA and may require a CD4/CD8 co-receptor involvement.

### 3.4 | p-i stimulation without eosinophilia

The absence of eosinophilia in some cases of DH may be linked to the involvement of CD4/CD8 co-receptors and/or low drug-TCR affinity.

Eosinophilia is observed in approximately half of the patients experiencing β-lactam antibiotic-induced DH. β-lactam-induced DHR can result from hapten or p-i mechanisms. For instance,
amoxicillin might act as a hapten-modified peptide, triggering T cells specific to the drug only in the presence of cofactors such as generalized virus infections (Figure 1A). In this case, the affinity of reactive T cells to the β-lactam-modified peptide may be low or normal, engaging CD4/CD8 co-receptors. Patients may exhibit mild clinical symptoms without eosinophilia. However, when amoxicillin acts via the p-i mechanism, certain p-i stimulations occur without CD4 or CD8 engagement, potentially increasing IL-5 production and leading to eosinophilia or even DRESS.

Abacavir, on the contrary, can cause severe DHRs without eosinophilia. In contrast to other drugs that elicit various p-i types, such as SMX, amoxicillin (Figure 1), abacavir binds with significant affinity exclusively to the HLA-B*57:01 pocket. This binding elicits a relatively monomorphic, exclusively CD8 T-cell response directed to the altered peptide and/or drug-modified HLA (Figure 1F). No other p-i stimulations are observed. Although abacavir TCCs exhibit high levels of IFNγ and cytotoxicity, IL-5 production is absent.

4 | SUMMARY AND CONCLUSION

Analysis of patients’ T cells directed to a great variety of drugs revealed that p-i stimulations are involved in most, if not all DRESS,
AGEP, SJS/TEN, and severe MPE cases. The term “p-i concept” encompasses various non-covalent drug bindings to immune receptors, known as “p-i stimulations” with a rather heterogeneous functional and, consequently, clinical outcome. Understanding these variations enables us to hypothesize that a specific type of p-i stimulation leads to an IL-5-TCR-signalosome and eosinophilia unique to DHR (DHR-EOS). This proposed mechanism is distinct from the antigen-driven, reactive forms with eosinophilic inflammation seen in atopic dermatitis or asthma: there tissue activation (TSLP, IL-25, eotaxins) precedes the development of Th2-based inflammation, which evolves under the influence of cytokines into a more Th2, IL-5 (and IL-13) secreting phenotype. It also aligns to the observation that DHRs, including IgE-mediated forms, are not more frequent in atopic diseases. The IL-5-TCR-signalosome hypothesis also differs from clonal expansions of eosinophils that lead to HES or L-HES (Table 1).

The generation of an IL-5-TCR-signalosome is not limited to DH. It may also occur in other high-affinity TCR- HLA interactions that do not involve the engagement of CD4 and CD8 co-receptors. Potential examples include superantigen-driven stimulations, where the superantigen bridges HLA with specific TCR Vβ; or in allo-stimulations such as graft-versus-host disease (GVHD), where a high-affinity interaction between an allo-allele and TCR occurs. In both conditions, T-cell stimulations that bypass CD4/CD8 co-receptor engagement, as well as eosinophilia, have been observed.

The formation of an IL-5-TCR-signalosome may occur in both p-i TCR and p-i HLA, as both types of p-i stimulations can result in low or high-affinity drug-TCR interactions. Analysis of TCCs elicited by lidocaine, sulfamethoxazole, and carbamazepine suggests that the drug can bind directly (Figure 1B) or additionally to the TCR (Figure 1E). Notably, high IL-5 levels have been repeatedly described in these TCCs. Furthermore, 3%–30% of these primarily CD4+ TCCs did not use the co-receptor CD4. P-i HLA types of p-i stimulations resulting in eosinophilia may be less frequent and represented by oxyurinol/allopinor induced DH (Figure 1D).

While the p-i stimulation and high IL-5 production by some drug-specific TCC is well established, making an “IL-5-TCR signalosome” as a concept quite likely, the proposed mechanism and involved components (lack of CD4/CD8 involvement, altered Lck/LAT activation) have not been formally proven and are rather hypothetical (Table 2: Figure 3). The topic of the TCR signalosome and the roles of its various components are quite complex. For instance, the function of Lck may depend on whether it is associated with CD4/CD8, bound to the cell membrane, or unbound. We are aware that further research is needed to understand how the IL-5 TCR signalosome leads to high IL-5 production.

Unfortunately, a comprehensive investigation of TCR signaling following p-i stimulation has not yet been conducted. Although there are data on Ca++ influx and early cell activation markers, such as annexin and CD69, most steps involved in the p-i-triggered TCR signalosome are unknown. Since many aspects of the TCR-signalosome are still unresolved, even in classical antigen reactions, investigations on labile drug-immune receptor bindings like p-i stimulations on the TCR-signalosome may have seemed premature. Additionally, DHRs are rare and unpredictable diseases, and the limitation to human cells may have delayed necessary studies.

The clinical implications of explaining EOS-DH through an IL-5 TCR signalosome are as follows:

- **Identifying DHR as p-i mediated**: Detection of p-i remains laborious and requires cell culture and functional assays. Screening for eosinophilia is part of the diagnostic work up for patients with DHR. Demonstrating eosinophilia may not only support the diagnosis of a DHR but may also suggest that the DH involves a (particular) p-i stimulation. Conversely, there are several p-i stimulations in which eosinophilia is absent: p-i stimulations are more frequent than eosinophilia in DH.

- **Targeting the beginning of a cascade** of events (T cells and IL5) could potentially block all sequential effects:
  - Corticoid-resistance: Eosinophilia in some DH-related conditions is not (or not sufficiently) responsive to corticosteroid therapy. Based on the IL-5-TCR signalosome hypothesis, it would be reasonable to evaluate therapies that more precisely interfere with the TCR signalosome, such as, for example, JAK inhibitors.
  - Anti-IL-5/anti IL-5R therapy has proven effective in severe allergic diseases, and improvement has also been observed in most DRESS patients with eosinophilia. This suggests a significant role of IL-5 in this form of DRESS.
  - Severity of DH: If, as proposed, the eosinophilia/IL-5 TCR signalosome results from a more affine TCR-drug/peptide/HLA interaction, the presence of eosinophilia may indicate that a longer-lasting T-cell stimulation takes place leading to more severe clinical
symptoms. Indeed, presence of eosinophilia in DHR was linked to a more severe clinical course.88

In summary, EOS-DH is NOT a result of an allergic process involving prior Th2 polarization and Th2 cytokine secretion, but may be connected to a specific p-i stimulation. One proposed possibility is that a high-affinity TCR–drug/peptide/HLA interaction enables bypassing CD4 co-receptors, leading to an altered Lck/LAT activation and resulting in high IL-5 production (IL-5-TCR signalosome). Eosinophilia based on a particular p-i stimulation distinguishes EOS-DH from allergic/atopic diseases or clonal forms of eosinophilia and may also explain the eosinophilia that can occur during GVHD and superantigen stimulations.

AUTHOR CONTRIBUTIONS
WJP created the concepts and formulated it; LT and DY contributed to the figures and formulations of the text.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Werner J. Pichler https://orcid.org/0000-0002-8117-359X
Lester Thoo https://orcid.org/0000-0001-9381-5943
Daniel Yerly https://orcid.org/0000-0003-2857-0737

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