Adverse Cutaneous Drug Eruptions: Review of Immunology, Pathogenesis, and Pharmacogenomics With Focus on HIV and TEN

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Adverse Cutaneous Drug Eruptions: Review of Immunology, Pathogenesis, and Pharmacogenomics with Focus on HIV and TEN

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Abstract:

Title: Adverse Cutaneous Drug Eruptions: Review of Immunology, Pathogenesis, and Pharmacogenomics with Focus on HIV and TEN

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Purpose: Immunologic principles behind antigen presentation inform the pathophysiologic basis of adverse cutaneous drug eruptions (ACDEs). We hypothesize that HIV predisposes to Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis (SJS/TEN), a severe ACDE, through decreased glutathione and regulatory T cells.

Methods: Pubmed literature was reviewed including MESH terms "Anti-HIV Agents" and "Drug Eruptions" with other controlled vocabulary, synonyms, and truncated terms in addition to Boolean operators for more comprehensive and unambiguous searches.

Results: HIV tropism for CD4+ in the circulating blood may decrease skin-directed CD4+, including CD4+ CD25+ T-regs that may prevent mild skin lesions from converting to TEN. HLA molecules play a crucial role in drug antigen presentation, and certain HLA alleles are present at higher frequency in patients who suffer from drug-specific ACDEs. Similarly, specific alleles related to glutathione synthesis, cytochrome P450 enzymes, and N-acetylatransferases important for drug metabolism appear in higher frequencies in patients with ACDEs and TEN induced by HIV-associated drugs.

Conclusions: HIV and HIV-associated medications predispose patients to SJS/TEN through different, likely interacting mechanisms. SJS/TEN pathogenesis is multifactorial and involves interplay of cytotoxic T lymphocytes and various cytokines and cell signaling pathways. HIV associated-medications increase susceptibility to SJS/TEN and ACDEs at different rates, possibly attributed to variable forms of drug metabolism and antigen presentation. Further study in the immunology and pharmacogenomics of ACDEs can identify appropriate screening

strategies, diagnostic biomarkers, and treatment targets, particularly in susceptible (e.g., virally infected) populations.

Work Description

My mentor and I brainstormed topics to review before initiating project. I designed the literature review, including organizational structure, research aims, and scope of the review. I learned and applied data search strategies, reviewed the literature using PubMed on chosen topics, and synthesized relevant and significant findings. I met with Carol Mita, Research Education Librarian at the Countway Library of Medicine. Together, we indexed MESH terms with controlled vocabulary, truncated terms and synonyms and additionally employed Boolean operators to ensure exhaustive and unambiguous searches. I also used reference lists of key articles for additional reading material. I used Zotero as a reference manager and systematically viewed titles, abstracts, and full texts. I wrote the entire manuscript and, after incorporating my mentor's feedback, prepared it for submission to Journal of Drugs in Dermatology for Continuing Medical Education article consideration on August 24, 2017.

Appendix:

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Introduction:

Adverse cutaneous drug eruptions (ACDEs) are drug reactions with poorly understood etiology and inadequate or controversial treatment options [1]. Although about 90% of ACDEs are benign[2], in severe cases (such as Toxic Epidermal Necrolysis (TEN)) they can be characterized by painful skin lesions, metabolic abnormalities, and a mortality rate of up to 30%. ACDEs also cost hospitals $5.6 million each year in the U.S. and can increase patient hospital stay by 5 days compared to patients with the same admission diagnoses[3]. The severe ACDE incidence rate in

AIDS patients is 1.0 per 1000 AIDS cases, while the incidence of severe ACDEs in the general population is about 1.2 per 1 million people, signaling a thousand-fold risk increase for ACDEs in AIDS patients[4]. Multiple synergistic and interacting factors might be attributed to the increased risk of ACDEs in AIDS patients, such as high drug dosages, altered cellular immunity, or HIV-associated infections. We review our current understanding of ACDEs with emphasis on TEN and its occurrence in HIV patients and drugs commonly prescribed to this population.

Overview of drug hypersensitivity immunology

Drug reactions are broadly categorized as type A and type B: type A reactions result from the known pharmacological action of the drug, while type B (idiosyncratic) reactions are more unpredictable[5]. Many type B reactions manifest in the skin and can be classified as immediate (IgE-mediated) or delayed (type IV hypersensitivity) as initially described by Coombs and Gell[6]. Many delayed cutaneous drug eruptions occur 1-3 weeks after drug exposure, but eruptions can occur within 2 days after drug re-exposure, suggesting an underlying immune mediated etiology in type B reactions.

The Hapten Hypothesis

Drugs may stimulate the innate immune system by acting as antigens interacting directly with toll like receptors (TLRs) on dendritic cells (DCs)[7], which may then upregulate the costimulatory molecule CD40[8,65]. DCs serving as antigen presenting cells (APCs) can then process antigens and present them as peptides to T cells. Drugs with lower molecular masses need to covalently bind to high-molecular weight proteins before they can act as antigens. Such a drug bound to a high-molecular weight protein (which would include self-peptides or human leukocyte antigen (HLA) molecules) is known as a hapten-carrier complex or drug-protein adduct. While some drugs (haptens) can directly bind to carrier proteins, other drugs must be

chemically modified first (i.e., pro-hapten to hapten transformation). The specific modification can determine which type of adaptive immunity is activated (e.g., humoral, cell, or both). Many drugs known to cause ACDEs have also been shown to form chemically reactive metabolites, including abacavir, which forms the metabolite aldehyde via alcohol dehydrogenase[9]. This process is called bioactivation.

Alternative models of antigen presentation

Certain drugs do not require the covalent binding described in the hapten hypothesis to stimulate the immune system. Termed the p-I concept, these drugs are said to have a pharmacological interaction directly with immune receptors (namely, TCRs)[8,10]. One way this was shown was by creating "T-cell clones" (TCC), which are drug-specific T-cells, or drug-specific peripheral blood mononuclear cells (PBMCs), extracted from patients allergic to both reactive and non-reactive (i.e., do not covalently bind to form hapten-carrier complexes) drugs [10]. Both reactive and nonreactive drugs increased intracellular calcium and downregulated TCR expression when added in solution with the drug-specific T-cell clones, suggesting drug recognition by T cells via a distinct mechanism. Another study suggested that the non-reactive drugs instead non-covalently bind to an MHC-peptide (multiple histocompatibility complex-peptide; another name for HLA) molecule to stimulate T cells as opposed to the covalent binding described in the hapten hypothesis[11]. Other experiments demonstrated TCC stimulation by nonreactive drugs with APC fixed to glutaraldehyde, suggesting that drug processing and protein covalent bonding by APC was not necessary. This mechanism has been implicated in several drugs, including carbamazepine[12], lamotrigine[13], mepivacaine[14].

Two other theories have described drug antigen presentation. The "Altered peptide Repertoire" model purports that the drugs themselves can also bind and modify self-peptides that

are presented to HLA molecules and TCR without directly binding to HLA[15]. The "altered TCR repertoire" model implies that the drug binds directly to TCR to alter its confirmation, which would subsequently allow TCR binding to a self-peptide-HLA complex.

Mechanism of Skin Hypersensitivity

Delayed-type hypersensitivity in the skin, also called ACDEs or cutaneous drug reactions (CDR), have been implicated in several drugs, including antimicrobials and NSAIDs[16]. Many studies have demonstrated an immunological component to ACDEs[17]. T cells specifically were implicated in one study where 17 of 22 patients with mild cutaneous drug eruptions demonstrated upregulation of HLA-DR on circulating CD4+ and CD8+ T cells[18]. The APCs in the skin are primarily Langerhan cells, which are responsible for recognizing haptens and subsequently migrating to lymph nodes to present haptens to T cells[19]. APCs also play a role in upregulating surface markers, such as MHC, costimulatory molecules, and various cytokines. As stated, in many cases the APC does not interact with the drug itself, but with a metabolite derivative once the drug undergoes bioactivation. While the liver is the primary site of bioactivation, the skin has been shown to harbor many enzymes required for metabolizing drugs, including cytochrome P-450[20] and cyclooxygenase[21]. Extracellular antigens travel via endosomes and undergo lysosomal degradation to be presented via MHC II (which includes HLA-DR, DP, and DQ) classically to CD4+ T cells, whereas intracellular antigens present with MHC I (HLA-A, B, and C) to CD8+ T cells.

Once the activated LC travels to the lymph node, the presented antigen-MHC complex must bind the TCR and a costimulatory receptor. LCs have been shown to express co-stimulatory peptides that specifically activate costimulatory receptors, including B7-1[22]. These costimulatory molecules are upregulated by cytokines (e.g., TNF-α, IL-1) released by skin cells upon exposure

to the drug[23]. The naïve T-cell then becomes clonally expanded into drug-specific T-cells with skin homing properties. T cells which ultimately travel to the skin have been shown to express a skin homing receptor called cutaneous lymphocyte-associated antigen (CLA), a carbohydrate epitope that binds to E-selectin. One study found CLA+ T cells in patients with nickel sensitivity activate when exposed to nickel, whereas CLA- T cells do not activate[24].

T cells can then secrete various cytokines, and the specific cytokine profile is a defining feature of drug-specific T-cells. ACDEs are considered type IV delayed hypersensitivity reactions which are divided into four main sub-types, each with its own major cytokine profile. Type IVa involves T helper 1 (TH1) cells, which secrete primarily IFNγ; type IVb involve TH2 cells, which secrete primary IL-4, IL-5, IL10, and IL13; type IVc cytokine profile is mediated by cytotoxic CD8+ T cells; and type IVd is mediated by chemokine ligand 8 and granulocyte-macrophage stimulating factor cytokines[25]. Immunohistological studies suggest different T cells contribute to the manifestations of ACDEs, including CD4+ and CD8+ T cells. Although overlap exists, the clinical presentation of the ACDE is associated with specific T cell activity. For example, CD8+ T-cell activity has been associated with more severe bullous skin disease[26], such as TEN[27]. It can thus be inferred that TEN predominantly displays a type IVc cytokine profile.

The role of Viruses in ACDEs

Patients with a viral infection have shown an increased rate of developing an ACDE[28,29]. In one study of 184 subjects with infectious mononucleosis, 45% of those receiving an antibiotic developed a rash compared to 16% of those receiving no antibiotic. Similar findings have been replicated in patients with HIV. One study of 684 HIV infected men reported 188 cutaneous reactions to drugs and visit rates for common inflammatory skin conditions was 5 times higher than those of non-HIV infected men[30], with trimethoprim-sulfamethoxazole (TMP-SMX) as the

most common likely culprit. Another interesting finding is that the very high rates of ACDEs from TMP-SMX in HIV patients has not been observed in HIV-seronegative patients in other immunosuppressed states[17]. In one study of patients receiving anti-tuberculosis medications, the relative risk of HIV co-infection was high among rifampin associated adverse drug reactions[31], suggesting a possible multifactorial synergistic effect leading to skin disease.

One hypothesis suggests that low glutathione levels in HIV patients predisposes them to ACDEs[32]. In one study, the total and reduced levels of glutathione in venous plasma of symptom-free HIV seropositive patients was 30% that of HIV-seronegative individuals[33]. Whether this decrease was due to decreased production or increased utilization was not determined. In another study, the blood of HIV patients demonstrated decreased glutathione levels and increased DNA fragmentation within lymphocytes[34], further substantiating that significant oxidative stress occurs during HIV infection. One study attempted to elucidate the role of epidermal keratinocytes in cutaneous drug hypersensitivity reactions focusing on the drugs dapsone and SMX[35]. These investigators found that normal human epithelial keratinocytes became susceptible to the toxicity of SMX-hydroxylamine (SMX-HA; a metabolite of SMX after bioactivation) only after prior depletion of glutathione and of dapsone without the need for prior glutathione depletion. This study suggests that reactive oxidative species formed by these metabolites may play a role in keratinocyte cytotoxicity. In another study, Dapsone-HA, which has previously shown greater toxicity than SMX-HA, generated higher ROS compared to SMX-HA, although there was not a simple correlation between ROS formation and cell death[36]. In these experiments, the presence of ROS scavengers played a significant role in ultimate ROS generation.

For drugs that are highly implicated in ACDEs in which the only indication is HIV infection, it is difficult to ascertain whether HIV itself is increasing the risk for ACDE. For example, in an international collaborative study, SJS/TEN (a severe ACDE) was shown in 18 of 246 HIV seropositive patients (7.3%); however, nevirapine was implicated in 15 of these cases[37]. Because ACDE has been reported in HIV seronegative patients receiving nevirapine as post exposure prophylaxis, this suggests that drug exposure to HAART may predispose to ACDE independently of HIV status.

Clinical Morphology of Cutaneous Drug Eruptions

ACDEs range in clinical severity and morphology. Milder reactions include maculopapular exanthem, which is commonly characterized by generalized erythematous macules or papules. Fixed drug eruption involves annular or oval erythematous macules and patches that reoccur in the same location after re-exposure to the same drug. Acute generalized exanthematous pustulosis (AGEP) is usually characterized by widespread non-follicular pustules localized in the epidermis. Drug reaction with eosinophilia and systemic systems (DRESS) is a rarer and potentially life threatening cutaneous drug eruption usually characterized by a diffuse, confluent erythema, facial edema, fever, lymphadenopathy, and internal organ involvement. There is also eosinophilia in 50 to 90 percent of cases and atypical lymphocytes in 30 to 70 percent of cases[38]. Finally, Stevens-Johnson syndrome/Toxic Epidermal Necrolysis (SJS/TEN) is characterized by diffuse erythema, painful skin lesions out of proportion to physical exam findings, and mucosal involvement[39]. Its course may last 8 to 12 days with persistent fever, epithelial sloughing, and can cause many complications associated with fluid loss through denuded skin, including hypovolemic shock and end-organ failure. SJS is defined as 10% or less body surface area (BSA) involvement, while TEN is defined as greater than 30% BSA affected.

Studies estimating TEN mortality vary; one systemic literature review found an overall percentage mortality of 30%, with risk factors including age, total BSA, and time to definitive treatment[40]. Associated clinical manifestations include mucositis, conjunctivitis, and involvement of the gastrointestinal, respiratory, and genitourinary tracts[1]. Given its high mortality and association with HIV, our review will focus on TEN.

TEN Pathogenesis

Toxic Epidermal Necrolysis, a severe type of ACDE, involves epidermal detachment with histopathological features including subepidermal blisters, keratinocyte apoptosis leading to erosions, and full thickness epidermal necrosis[41]. Drugs are the most common cause of TEN and over 100 drugs have been implicated, with some of the more common including anticonvulsants, oxicam-non-steroidal anti-inflammatory drugs, allopurinol, sulfonamide antibiotics, corticosteroids, and nevirapine[42,43]. Genetic risk factors for TEN include the frequency of drug-specific HLA alleles in certain populations (discussed in Pharmacogenomics section)[44], while non-genetic risk factors may include underlying malignancy, co-administration of anti-cancer agents[45], and other systemic diseases, such as systemic lupus erythematosus.

T cells help regulate keratinocyte cytoxicity and have been demonstrated to play a role in the pathological mechanism of TEN[1]. It has been proposed via the "altered TCR" repertoire model that the culprit drug can bind TCR and HLA to form an HLA-TCR-drug complex which results in expansion of CD8+ cytotoxic T lymphocytes (CTL). The expansion of CD8+ CTL occurs predominantly in the peripheral blood[46]. CD8+ CTL subsequently carry out the immune reactions in an MHC I-restricted manner that lead to keratinocyte death in TEN[12,15,47]. One study identified that blister lesions in SJS-TEN patients contained both CTL and natural killer (NK) cells.

The subsequent action of CTL and NK is elucidated via the various cytotoxic proteins they secrete. Quantitative PCR and immunohistochemistry studies demonstrated that the protein granulysin was the cytotoxic molecule of highest expression in the blister fluid[48]. Studies with human and mice cells showed that CD8+ CTL and NK cells trigger keratinocyte apoptosis via granulysin release and that depletion of granulysin in blister fluid of TEN patients reduced the amount of apoptosis. Granulysin serves several functions, including acting as chemoattractant for T cells, increasing expression of chemokines and cytokines, and promoting APC recruitment by acting as an alarmin. In addition to granulysin, perforin and granzyme B are two other cytolytic proteins secreted by CD8+ CTL and NKs, and increased levels of these two proteins correlated to disease severity in ACDEs[49]. The death ligand TRAIL (TNF-related apoptosis-inducing ligand) was also found in greater amounts in TEN blister fluid than in TEN sera or normal sera[50]. TRAIL was found to induce keratinocyte death in vitro and was secreted by CD1a+ and CD14+ cells inside blister fluid, suggesting a mechanism of cell death independent of MHCI/CD8+ T cells.

Death receptor Fas/CD95 and Fas ligand (FasL) have also been implicated as a possible trigger for keratinocyte apoptosis[51]. This was shown in a study where soluble FasL had increased expression in serum of TEN patients, and FasL had increased expression in skin biopsies of TEN patients, suggesting that the increase in the sera was a result of FasL cleavage on the membrane of epidermal cells[52]. Interestingly, though, one study showed inhibition of perforin/granzyme B via EGTA or Concanamycin A decreased CTL mediated apoptosis of keratinocytes, whereas addition of an anti-Fas monoclonal antibody did not decrease cell lysis, although this was only observed in the blister fluid of one clotrimoxazole-induced TEN patient[53]. Another study showed that keratinocyte FasL was predominantly intracytocplasmic in vivo and could not initiate

apoptosis under physiological conditions[54]. Therefore, the exact role of Fas/FasL in TEN remains somewhat controversial.

Many cytokines have been linked with TEN; in one immunohistochemical study, biopsies of bullous lesions in TEN patients showed a dense layer of TNF-α[55], and another showed significantly higher expression of (TNF)-α and interferon (IFN)-γ in biospies of TEN patients compared to controls[56]. These cytokines may promote various overlapping processes, such as apoptosis, necrosis, lymphocyte recruitment, radical formation, and inflammatory responses/endothelial permeability. More recently, the supernatant from PBMCs exposed to the causative drug in TEN patients induced keratinocyte death, and mass spectrometry identified the protein annexin1 as a key mediator, with its depletion failing to lead to keratinocyte death[57]. TEN keratinocytes were shown to express FRP1, the receptor for annexin 1, and their interaction induced necroptosis, or programmed cell necrosis. Receptor-interacting protein kinase-3 (RIPK3) executes necroptosis and is highly upregulated in the skin of TEN patients in addition to phosphorylated mixed lineage kinase domain-like protein (MLKL), a downstream component of RIPK3[58]. Dabrafenib, a RIPK3 inhibitor, prevented MLKL phosphorylation and decreased cell death in an in vitro model, suggesting a potential future treatment option. PCR analysis has recently shown that microRNA 18a-15p levels were increased in TEN patients in vivo and transfection of this miRNA in vitro increases apoptotic activity[59]. In one study, IL-15 and granulysin were significantly correlated with disease severity, and IL-15 levels were associated with increased mortality in 112 patients of SJS/TEN and enhanced cytotoxicity of cultured NK cells of TEN patients[60]. Despite advancement in understanding, SJS/TEN is clearly a complex phenomenon and its exact mechanism remains unknown. Mechanistic understanding can help

uncover possible biomarkers (e.g., granulysin, FasL) for earlier diagnosis, but measuring these biomarkers can be costly and is not currently clinically utilized[61].

A notable challenge in studying TEN and other cutaneous drug reactions is "proving" the causative agent. Systemic re-exposure of the possible agent can be dangerous for patients. Patch testing can produce delayed T-cell hypersensitivity but is known to have poor sensitivity[62]. In one recent study, a patient with bacteremia treated with multiple antimicrobials subsequently developed TEN, and to find the culprit antimicrobial, investigators incubated samples of each antimicrobial overnight and measured the IFN-γ release of PBMCs by enzyme-linked immunospot assay (ELISpot)[63]. Measuring upregulation of CD137, a TNF receptor family member, by flow cytometry was used as a proxy for T-cell activation on CD3+CD8+ T cells. The IFN-γ ELISspot assay 4 days after TEN diagnosis demonstrated a positive response to only one antimicrobial, teicoplanin, and only teicoplanin upregulated CD137 at this time point. Notably, teicoplanin responses were absent 5 and 8.5 months later both in ELISpot and patch testing, demonstrating the diagnostic importance of acute testing. Teicoplanin also shared the highest ALDEN (algorithm of TEN drug causality) with another antimicrobial in this case, which would have also been considered the causative drug and again highlights the often-ambiguous nature of ascribing causality.

TEN and HIV Patients

Drugs predisposing to TEN that are often prescribed to HIV patients include antiretroviral, antituberculosis, and sulfa-containing drugs, but HIV may predispose to TEN independently of these agents. Regulatory CD4+ cells, which are a type of regulatory T-cell (Treg) lymphocyte have been shown to suppress the immune response and were able to prevent TEN in transgenic mice when co-transferred I CD11c+ dendritic cells[64]. In dermis biopsies of

patients with ACDE, the number of CD4+ cells is decreased[65], and although most CD4+ cells are *memory* T cells, a significant amount of CD4+ cells in these samples co-express CD25+ and FoxP3, which are markers for *regulatory* T-cells (Tregs). Treg frequency in early and late-stage samples of SJS/TEN appear to be stable, but non-Treg cell frequency was increased when the SJS/TEN was resolving[66]. Another sample of skin biopsies of TEN patients demonstrated an increased ratio of CD8 (+) to CD4+ cells in the dermis by 8-fold[67] compared to non-HIV infected TEN patients. The HIV skin samples also showed a decreased CD25+ to CD4+ ratio in the epidermis and a trend towards decreased CD25+ cells in the dermis. In all, these results suggest that HIV tropism for CD4+ in the circulating blood may also affect skin-directed CD4+, including CD4+ CD25+ T-regs.

It is thus hypothesized that CD4+ Tregs may help overcome CD8+ cytotoxic T cell activity and prevent mild skin lesions from converting to more severe TEN, though antigen presentation may be required given that Tregs only prevented epidermal injury in combination with CD11c+[54]. The skin has a separate immune system independent of peripheral blood circulation with approximately 20 billion resident skin T cells. It is unclear whether decreased CD4+T regs in ACDE patients is attributed to problems in Treg blood to skin migration or problems inherent in Treg skin proliferation.

Pharmacogenomics of ACDEs

Pharmacogenomic studies have identified single nucleotide polymorphisms (SNPs) in HLA alleles and alleles related to drug metabolism that are present at a higher frequency in ACDEs induced by specific drugs and sometimes in specific populations. We will consider genetic associations of ACDEs and TEN specifically with antiretroviral and non-antiretroviral drugs commonly prescribed to HIV patients.

HAART - Abacavir

Abacavir is a nucleoside reverse transcriptase inhibitor highly efficacious for HIV-1, but up to 14% of exposed patients have been shown to develop hypersensitivity[68]. Although abacavir induced drug hypersensitivity is common, there are few reports of abacavir-associated SJS/TEN[69]. Given the pathogenesis of ACDEs likely involving MHC-restricted presentation of drug or drug metabolites to T cells and that familial disposition had been implicated in abacavir-induced ACDEs, examiners sought to find specific MHC alleles in 200 Western Australian HIV patients with abacavir hypersensitivity[70]. The HLA-B*5701 allele was present in 78% of patients who experienced abacavir hypersensitivity and in 2% of abacavir tolerant patients, while the HLA-DR7 and HLA-DQ3 combination was present in 72 and 3% of abacavir sensitive and abacavir tolerant patients, respectively. Screening patients for HLA-B*5701 successfully eliminated abacavir induced hypersensitivity reaction in a double-blind, prospective, randomized study with 1956 HIV infected patients taking abacavir for the first time[71], and the allele had a 100% negative predictive value (i.e., patients without the allele did not develop hypersensitivity). The results were replicated in other populations, including Caucasian adults[72] and Thai and Cambodian children[73]. These results, in addition to the availability of cost-effective lab testing, allowed the successful implementation of genetic screening for abacavir sensitivity.

Experimenters have shown that abacavir follows the "altered peptide repertoire" model, where abacavir itself binds to HLA-B*57:01 in a non-covalent fashion, changing the conformation of the antigen-binding cleft and altering the repertoire of endogenous peptides that can bind to HLA-B*57:01[74]. These endogenous "self" peptides are recognized as foreign and activate cytotoxic CD8+ T cells to induce abacavir hypersensitivity. In vitro PBMC cultured with abacavir leads to CD8+ T cell activation independent of costimulatory signals (DC maturation or

release of inflammatory cytokines was observed)[75]. Abacavir undergoes bioactivation into abacavir-aldehyde, which may be detoxified by glutathione conjugation[76]. This detoxification mechanism may be deficient in HIV patients, although no conclusive evidence exists. Abacavir-specific T cells had decreased reactivity when transporter associated with antigen processing (TAP) was inhibited and TAP-associated glycoprotein (tapasin) was absent, further suggesting that abacavir presentation and hypersensitivity depend on MHCI[77].

Immunologically confirmed abacavir hypersensitivity can occur less than 48 hours after a patient's first exposure to the drug, suggesting that abacavir in certain cases may be predominantly stimulating a memory T-cell population rather than a naïve-T cell population[78]. Notably, abacavir in one study appeared to induce T cells from both memory and naïve compartments[79]. Abacavir reactive CD8+ responses were recorded in vitro from the blood of 100 HLA-B*57:01 healthy patients using the ELISpot assay[69], highly suggesting pre-existing abacavir-reactive memory CD8+ T cells likely from exposure and cross-reactivity to previous foreign antigens. More recently, abacavir was found to release IL-1β from human monocytes inhibited with NLRP3 knockdown, suggesting a role for abacavir in NLRP3 inflammasome activation. IL-1β release and processing correlated with mitochondrial reactive oxygen species creation, and mitochondrial ROS production was also mitigated by NLRP3 knockdown. HSP70, which plays a role in chaperoning HLA I presentation and innate immune response initiation, was shown to be localized inside APCs after abacavir induction, and increased levels of HSP70 were found in abacavir-hypersensitive compared to abacavir-tolerant controls[80]. The Hsp70 493T allele has also been shown to predict abacavir hypersensitivity[81]. These studies highlight that innate immune activation may play a role in delayed-type hypersensitivity, particularly in abacavir where hypersensitivity can occur relatively quickly.

HAART - Nevirapine

Nevirapine in combination with 2 nucleoside reverse transcriptase inhibitors is commonly used as first line treatment for HIV infection in developing countries, but it is unfortunately highly associated with TEN/SJS and hepatoxicity. Nevirapine-induced hypersensitivity occurs 14 to 21 days after drug administration and occurs faster upon re-challenge, suggesting an immunological mechanism[82]. Skin hypersensitivity in nevirapine appears to be caused by 12-sulfonyl-nevirapine (NVP), which is derived via sulfonation of 12-OH-NVP, a drub metabolite of nevirapine. This was first implicated with experiments where treating mice with 12-OH-NVP caused rash but inhibiting 12-OH-NVP formation did not cause rash[83]. In another experiment, inhibition of sulfonation of 12-OH-NVP prevented rash in human skin[84]. Interestingly, a lower pre-treatment CD4+ T cell count is protective against rash and hepatitis; these reactions are more common and severe in non-HIV infected individuals receiving prophylactic nevirapine[85]. This may suggest that certain HLA markers located within the class II regions of the MHC may increase susceptibility to an immune response against nevirapine specific antigens via CD4+ T cells.

In one attempt to study this, investigators found that the HLA-DRB1*0101 allele in combination with a higher percentage of CD4+ T cells was associated with hepatic/multisystem nevirapine reactions in a cohort of 235 Western Australian HIV patients[86]. In a smaller study of 49 HIV Sardinian patients, 6 of 13 (46%) patients who developed hypersensitivity to nevirapine had the HLA-Cw8 and HLA-B14(65) antigens compared to 2 of 36 (5%) of the group who did not develop a reaction[87]. HLA-Cw8 was further implicated in a study of 41 HIV-1 infected Japanese patients who received Nevirapine where 42% of the 11 patients with nevirapine hypersensitivity were HLA-Cw8 positive compared to only 10% of the 29 nevirapine-tolerant

patients[88]. In a Thai population, the HLA-B*3505 allele was observed in 17.5% of 147 nevirapine-induced skin rash HIV patients compared to 1.1% of 185 nevirapine-tolerant HIV patients[89]. It appeared, then, that MHC class I and CD8+ T-cell mediated immune responses also played a role in nevirapine hypersensitivity.

Further studies tried to distinguish allele association of MHCI and II with hepatic and cutaneous events. One study identified that that HLA-B*35:01 and HLA-Cw4 were associated with cutaneous nevirapine hypersensitivity, HLA-DRB1*01:01 was associated with hepatitis, and that nevirapine-specific IFNγ responses measured via ELISpot assay were completely diminished when CD4 or CD8+ T cells were depleted from PBMC, suggesting that both CD4 and CD8+ nevirapine-specific T cells are involved in the immuno-pathogenesis.

Previous studies characterized the biotransformation of nevirapine as involving CYP450 metabolism, glucuronide conjugation, and urinary excretion[90]. Drug metabolism and transport genes were studied in addition to MHC in a case-control trial which showed that CYP2B6 516GT was associated with nevirapine-induced cutaneous events in HIV patients of African, Asian, and European descent[91]. This study also showed an association between HLA-Cw*04 and cutaneous events in all three populations, HLA-B*35 and cutaneous events with an Asian population, and HLA-DRB*01 and hepatic events with a White population, although it is important to consider that lack of significant associations may be attributed to low frequency of the alleles in certain populations. These studies support that the pathogenesis in nevirapine hypersensitivity involves a cutaneous component likely mediated by CYP2B6 metabolism and CD8+ MHCI-specific immune responses with an additional hepatic component more strongly mediated by a CD4+ MHCII-specific immune response. A more recent study found an association in a South African HIV population between nevirapine associated hepatic events and

both HLA-B*5801 and HLA-DRB1*0102[92], so the hepatic and MHCII association may not be true for all populations.

With regards to SJS/TEN specifically, like previously studied nevirapine hypersensitivity phenotypes, SJS/TEN were associated with HLA C*04:01 in a Malawian population[93]. One study identified CYP2B6 SNPs highly associated with nevirapine-induced SJS/TEN in a Mozambique population, with the 983C allele associated with higher risk and the 516G and 983T haplotype showing a protective effect[94]. This result was later replicated in a Malawian and Ugandan population[95]. HCP5, which encodes endogenous retroviral elements, was associated with SJS/TEN susceptibility in 27 Mozambique HIV patients and 76 controls[96], but its role in pathogenesis remains unclear. Variants of the TRAF31P2 gene, which encodes for TRAF proteins that play a role in TNF receptor signaling pathways, have been associated with increased susceptibility to nevirapine-induced SJS/TEN[97], although the study only included 27 Mozambican patients with SJS/TEN. Polymorphisms of glutathione S-transferases, enzymes crucial for cellular detoxification and reducing oxidative stress, found that the GSTM1 null genotype was present at higher frequency in nevirapine-induced SJS/TEN patients[98]. In a previous study, it was shown that GSTM1 can conjugate 12-sulfoxyl-NVP, the nevirapine metabolite implicated in skin hypersensitivity[99]. GSTM1 null genotype may thus lead to decreased rate of enzymatic reaction and buildup of 12-sulfoxyl-NVP. In all, these studies demonstrate that multiple pathways in nevirapine presentation and metabolism contribute to the SJS/TEN phenotype.

Non-HAART: Sulfamethoxazole

Sulfonamide antibiotics (sulfamethoxazole (SMX), clotrimoxazole (TMP-SMX), sulfadiazine, sulfasalazine, sulfadoxine) are used to treat Pneumocystis *jirovecii*, toxoplasma

encephalitis, Isospora, and other infections in HIV patients[100]. Sulfonamide pathogenesis involves SMX bioactivation to SMX-HA in various places, including in the liver via CYP2C6/9[101] and in phagocytic cells by myeloperoxidases (MPO)[102]. SMX-HA then spontaneously oxidizes to SMX-nitroso (SMX-NO), which can then become a drug-protein adduct acting as a hapten presented by DCs to T cells with MHCI or MHCII. DCs highly express CD40 when exposed to SMX-NO, a costimulatory molecule that propagates T cell activation[103]. This activation is thought to be responsible for sulfonamide hypersensitivity. SMX hypersensitivity affects about 3% of the population and is one of the most common drugs associated with SJS/TEN[104]. A case-control study found that the relative risk with TMP-SMX was 172 for developing SJS/TEN[41]. Clotrimoxazole hypersensitivity occurs at a rate of 8% in HIV-negative individuals but ranges from 20-100% in patients with AIDS[105].

Unlike nevirapine and abacavir, sulfonamide-induced SJS/TEN had only a weak association with an HLA allele (namely, HLA-B*38) in European patients[106]. Low CD4[107] but a high CD4:CD8 ratio[108] have been associated with increased risk. Molecular dynamic studies show that the CDR2β loop within the Vβ20-1 domain of TCR binds to SMX with high affinity, which subsequently bind HLA-peptide complexes with higher affinity[109].

With regards to drug metabolism, there is controversial evidence regarding the role of N-acetyltransferases (NAT) predisposing to SMX hypersensitivity. NATs detoxify the parent drug into an inactive metabolite that can be excreted in the urine, and polymorphisms in NAT2 gene lead to “slow N-acetylation”, which are more common in patients with SMX hypersensitivity[110] and SJS/TEN[111]. However, one study of 99 Caucasian adults showed no association with NAT2 gene polymorphisms that lead to slow acetylation phenotype and sulfonamide hypersensitivity compared to controls[112]. A recent Genome Wide Association Study (GWAS) substantiated the

negative result in immunocompetent patients[113]. The NAT2 slow acetylator genotype was also not associated with clotrimoxazole hypersensitivity in a cohort of HIV positive patients[114]. Interestingly, gain of function alleles in NAT1 was shown to be protective against SMX-hypersensitivity in a cohort of HIV patients, but this was shown only in HIV patients with NAT2 slow acetylator polymorphism, suggesting a gene-gene interaction[115].

As stated, glutathione levels have been shown to be decreased in HIV patients[31]. Glutathione (GSH) bioinactivates SMX-NO into SMS-HA, and glutathione deficiency has been proposed as a contributing factor to sulfonamide hypersensitivity by leading to increased amount of toxic SMX-NO[116]. GSH has been previously shown to decrease SMX cytotoxicity[117] in vitro. Glutamate cysteine ligase catalytic (GCLC) subunit is the rate limiting enzyme in GSH biosynthesis, and a certain SNP rs761142 G located within intron 1 of GCLC was associated with reduced GCLC expression in B lymphocytes and SMX hypersensitivity in HIV/AIDS patients[118]. Notably, the study did not control for comorbidities or co-medication administration, which theoretically can affect the level of other toxic metabolites, the rate of SMX bioactivation/inactivation and thus the rate of SMX hypersensitivity.

Conclusions

The etiology of ACDEs is incompletely understood but is thought to involve immunological components given that they occur more rapidly after drug re-exposure. Drugs undergo covalent and non-covalent chemical modification with HLA molecules, while other drugs modify self-peptides and TCRs before they are presented to T cells. Drug presentation occurs through Antigen Presenting Cells (APCs), and Langerhan cells are the primary APC in the skin, upregulating HLA, costimulatory molecules, and cytokines during drug presentation. Once drugs are presented, certain receptors on T cells help generate skin-specific drug

hypersensitivity reactions, such as cutaneous lymphocyte-associated antigen, by promoting T cell travel to skin. T cells then secrete different cytokines that play a key role in the clinical manifestations of the ACDE. Importantly, drugs may undergo bioactivation through various enzymes, forming metabolites that may ultimately interact with APCs and HLA molecules. The decreased degradation of these metabolites may explain why patients with underlying viral infection have increased susceptibility to ACDEs; specifically, patients with HIV have lower glutathione levels, so metabolites may build up and have higher propensity for oxidative stress, generating clinically significant ACDEs.

Toxic Epidermal Necrolysis (TEN) is an ACDE of particular interest given its morbidity, mortality, and lack of viable treatment options. Drugs implicated in TEN bind to TCR and HLA to cause expansion of CD8+ cytotoxic T-lymphocytes (CTL) cells, triggering keratinocyte death partially through secretion of granulysin and other cytotoxic proteins. Multiple signaling pathways have been implicated, involving TRAIL (TNF-related apoptosis-inducing ligand) and Fas/Fas ligand. Evidence supports (TNF)-α and interferon (IFN)-γ as major cytokines involved in TEN, and more recent discoveries have revealed many other mediators, including annexin 1, RIPK3, and microRNA 18a-15p. Further understanding in underlying pathophysiology can inform future drug targets in preventing TEN; the RIPK3 inhibitor dabrafenib, for example, was shown to decrease apoptotic activity in an in vitro model. The pathophysiology is influenced by the causative drug, which can sometimes be difficult to identify. Newer tests identifying diagnostic biomarkers, such as the IFN-γ ELISspot assay, show promise in accurately finding the drug culprit in TEN. Co-existing HIV may also contribute to the pathophysiology of TEN through decreased number of regulatory T cells.

Drug presentation and metabolism may vary significantly based on single nucleotide polymorphisms (SNPs) in a person's genome. The HLA-B*5701 allele was more commonly found in patients with abacavir hypersensitivity, prompting the current use of genetic testing as an effective screening tool. This allele association suggests CD8+T cell/MHCI mechanism, but other studies implicate HSP70 and activation of innate immunity, which may explain the rapid onset of abacavir hypersensitivity. Nevirapine hypersensitivity, by contrast, has shown associations with certain alleles of both MHCI and II and CYP2B6, a drug metabolizing enzyme. Certain alleles increase susceptibility to nevirapine induced SJS/TEN, including GSTM1 involved in glutathione conjugation of nevirapine metabolites. Sulfonamides have a weak association between HLA alleles and developing SJS/TEN. Like all three drugs, sulfonamides form metabolites that are bioinactivated by glutathione, substantiating the role glutathione deficiency may play in increasing susceptibility to SJS/TEN in HIV patients. Pharmacogenomic associations highlight the importance of studying ACDEs induced by different drugs as separate etiological entities and necessitates further study in drug-specific metabolic and immunologic presentation pathways.

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