Allopurinol-induced severe cutaneous adverse reactions: A report of three cases with the HLA-B*58:01 allele who underwent lymphocyte activation test

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Allopurinol is frequently used to treat hyperuricemia. However, allopurinol may induce severe cutaneous adverse reactions (SCARs); these life-threatening disorders include Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome. Associations between human leukocyte antigen (HLA) genotype and drug-induced hypersensitivity to abacavir, carbamazepine, and other drugs have been described.[2,3] Allopurinol-induced SCARs have been reported in various ethnic populations, including Chinese,[4] Japanese,[5] and Korean.[6] We describe our experience with three patients who developed allopurinol-induced SCARs; we review the clinical findings, HLA-B genotypes, and results of lymphocyte activation test (LAT).

Written informed consent was obtained from all three patients and the work was approved by the Institutional Review Board of Inje University Busan Paik Hospital, Busan, Republic of Korea.

We collected clinical data, HLA-B genotyping results, and LAT outcomes for all patients.
**HLA genotyping**

Peripheral blood was collected from all patients, and genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using a QIAamp® Blood Mini Kit (QIAGEN, Hilden, Germany). We determined HLA-B genotypes using polymerase chain reaction (PCR)-sequence-based typing (SBT) (SBT Engine software version 2.20; GenDx, Utrecht, the Netherlands).

**LAT**

To explore drug hypersensitivity, we subjected PBMCs to LAT. We also incubated with or without 0.5 µg/mL phytohemagglutinin A (PHA; positive and negative controls, respectively; Sigma-Aldrich, St. Louis, MO, USA). Briefly, we subjected allopurinol, carbamazepine, and phenytoin to LAT. Blood was layered onto Histopaque®-1077 (Sigma-Aldrich) and single-cell suspensions were obtained by centrifugation. These suspensions were added to 96-well culture plates and stimulated with several concentrations of allopurinol for 48 and 72 h. The allopurinol concentrations used were the same as those in a case report by Kim et al.[8] After incubation, the CD69 and CD25 expression levels on CD4+/CD8+ T cells were measured by flow cytometry.[9] The cells were harvested and stained with anti-human CD3 PE, anti-human CD4 PECy5, anti-human CD8 APC, anti-human CD69 FITC, and anti-human CD25 PECy7 (eBioscience, San Diego, CA, USA). Data acquired with the aid of a FACSCTo™ II flow cytometry (BD Biosciences, San Jose, CA, USA) were analyzed using FlowJo® software (Tree Star, Ashland, OR, USA). The data from stimulated samples were divided by those from unstimulated samples to obtain stimulation indices (SIs). An SI > 2.0 was interpreted as positive.

**Case Reports**

**Patient 1**

A 63-year-old male presented with a 14-day history of pruritic erythematous papules over his entire body, accompanied by jaundice. He had been using a topical corticosteroid and had taken an antihistamine for 10 days before visiting, but the symptoms became aggravated, with eye and oral mucosal involvement. To treat hypertension and gout, he had commenced anti-hypertensive drugs 5 months prior and allopurinol 3 weeks prior, respectively. A high fever (>38.0°C) was evident on physical examination. Laboratory tests revealed elevated levels of aspartate aminotransferase (AST; 161 U/L), alanine aminotransferase (ALT; 390 U/L), total bilirubin (10.9 mg/dL), and eosinophils (2,432/mm³). The histopathological data were compatible with erythema multiforme. Genetic analysis revealed the HLA-B*13:02/*58:01 genotype. We diagnosed DRESS syndrome caused by allopurinol and prescribed systemic corticosteroids, after which clinical improvement was evident.

**Patient 2**

A 71-year-old female presented with a 3-day history of painful erythematous scaly patches that were coalescing into large patches, progressive desquamation over her entire body, a febrile sensation, and general weakness. She had been taking anti-hypertensive medication for 5 years. One month prior, she had commenced allopurinol and colchicine to treat newly diagnosed gout. Her body temperature was >38.0°C. Laboratory tests revealed elevated levels of AST (281 U/L), ALT (118 U/L), and eosinophil (1,607/mm³). The histopathological data were compatible with erythema multiforme. Genetic analysis revealed the HLA-B*48:01/*58:01 genotype. We diagnosed DRESS syndrome attributable to allopurinol. She was also diagnosed with end-stage renal disease (ESRD) during work-up of the skin lesions. After 3 months of treatment with systemic and topical corticosteroids, clinical improvement was evident.

**Patient 3**

A 22-year-old male presented with a 10-day history of painful

![Figure 1. Patient 3: Erythematous macules with edema on the face (A); erythematous macules on the upper and lower extremities (B-C); and lymphocyte infiltration evident on skin biopsy (D-E).](image-url)
erythematous scaly patches coalescing into larger patches, pro-
gressive desquamation over the entire body, marked facial swell-
ing, and a febrile sensation (Fig. 1). He had a history of diabetes mellitus, tuberculosis, and gout. He had taken anti-hypertensive
drugs for 5 years, and had commenced anti-tuberculosis medi-
cation 4 months prior, and allopurinol 1 month prior (to treat
gout). High fever (>38.0°C) and submandibular lymph node
enlargement were evident on physical examination. Laboratory
tests revealed elevated levels of AST (86 U/L), ALT (101 U/L),
and eosinophils (2,239/mm³). Histopathological examination
revealed acanthosis with severe lymphocytic exocytosis and
spongiosis of the epidermis, and dense perivascular and peri-
follicular lymphocytic infiltration (Fig. 1). Genetic analysis re-
vealed the HLA-B*44:03/*58:01 genotype. LAT was positive for
allopurinol (Fig. 2). He was diagnosed with DRESS syndrome
attributable to allopurinol and treated with cyclosporine, anti-

Figure 2. An allopurinol-positive lymphocyte activation test (stimulation index >2). CD69 was upregulated by allopurinol in CD4⁺ T cells from Patient 3 at both 48 and 72 h after culture. Sequence: negative control; various drug concentrations, and the phytohemagglutinin A (PHA) positive control.

Table 1. Clinical and laboratory characteristics of patients with allopurinol-induced SCARs (all had DRESS syndrome)

| Patient | Gender / age (years) | Allopurinol dose (mg/d)/ duration (d) | Sx. | Underlying disease | HLA-B | LAT |
|---------|---------------------|--------------------------------------|-----|--------------------|-------|-----|
| 1       | M/63                | 100/14                               | 7   | Hypertension       | *13:02, *58:01 | -   |
|         |                     |                                      |     | Gout               |       |     |
| 2       | F/71                | 200/30                               | 24  | Hypertension       | *48:01, *58:01 | -   |
|         |                     |                                      |     | ESRD               |       |     |
|         |                     |                                      |     | Gout               |       |     |
| 3       | M/22                | 200/2                                | 20  | DM                 | *44:03, *58:01 | +   |
|         |                     |                                      |     | Tuberculosis       |       |     |
|         |                     |                                      |     | Gout               |       |     |

SCARs, severe cutaneous adverse reactions; DRESS, drug reaction with eosinophilia and systemic symptoms; HLA, human leukocyte antigen; LAT, lymphocyte activation test; ESRD, end-stage renal disease; DM, diabetes mellitus. *Symptom onset after starting the drug.
Discussion

We diagnosed three patients with allopurinol-induced SCARs based on clinical features, pathological findings, and laboratory data; all had DRESS syndrome. All patients were taking allopurinol to treat gout and recovered from their allopurinol-induced SCARs after intensive care. All had HLA-B*58:01 alleles and LAT confirmed that allopurinol was the causative agent in one patient (Fig. 2). The patients had taken allopurinol at 100–200 mg/d for 2–30 days; the time to symptom onset after the initial dose was 7–24 days (Table 1). Allopurinol-induced hypersensitivity often co-exists with an HLA-B*58:01 allele and renal impairment.[10] Patient 2 had both an HLA-B*58:01 allele and ESRD, triggering DRESS syndrome. The other patients also had concomitant diseases: hypertension, diabetes mellitus, and tuberculosis.

Genetic markers apart from HLA-B*58:01, including HLA-C*03:02 and A*33:02 (risk factors) and A*02:01 (a preventative marker), are reportedly associated with allopurinol-induced SCARs in Korean patients.[6] We performed HLA-B genotyping only; we do not know whether the patients had other HLA risk alleles. Jutkowitz et al. found that HLA-B*58:01 testing before allopurinol prescription was cost-effective for Asians living in the USA (such as those of Korean, Thai, or Han Chinese descent),[11] and also when the population frequency of HLA-B*58:01 was >1.6%. The HLA-B*58:01 allele frequency in Koreans is 6.77%,[12] rendering this allele relatively common; it may be cost-effective to evaluate HLA-B*58:01 status before prescribing allopurinol.

LAT serves to detect drug sensitization at the cellular level in patients with non-immediate T cell-mediated drug hypersensitivity reactions; the sensitivity and specificity of LAT are 16.7% and 97.8%, respectively,[7] suggesting that LAT can be used to confirm a causative agent. Only 1 of the 3 patients was positive on LAT because of the low sensitivity of the test; however, the result was meaningful in that the causative agent was identified. Other diagnostic tests for non-immediate drug hypersensitivity reactions include the in vitro lymphocyte transformation test (LTT) and enzyme-linked immunospot (ELISPOT) assays; and in vivo delayed-reading intradermal tests, patch tests, and provocation tests.[7] LAT has an advantage of higher specificity than the in vivo tests, and is more practical. However, neither LAT nor the LTT is sensitive; this problem can be addressed by combining such tests with assays of drug-specific cytokine levels.[7,13] We performed LAT only.

The association between the HLA-B*58:01 allele and allopurinol-induced SCARs is well known, but the underlying mechanism remains unclear. We found that one patient reacted to allopurinol upon LAT. HLA-B genotyping would aid in prevention of allopurinol-induced SCARs, and LAT can serve to identify the causative agent. Further development of diagnostic methods is needed to provide a cost-effective, rapid, and accurate method predictive of the development of allopurinol-induced SCARs.

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Conflict of interest

No author has any conflict of interest relevant to this study.

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