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NK cells are biologic and biochemical targets of 6-mercaptopurine in Crohn's disease patients

Susy Yusung [a,*], Dermot McGovern [b], Lin Lin [e], Daniel Hommes [c], Venu Lagishetty [c], Jonathan Braun [d]

[a] Department of Pediatrics, Harbor UCLA Medical Center and Los Angeles Biomedical Institute, Torrance, CA, United States
[b] Translational Genomics Group, F. Widjaja Foundation Inflammatory Bowel and Immunobiology Research Institute, and Medical Genetics Institute, Cedars Sinai Medical Center, Los Angeles, CA, United States
[c] Division of Digestive Diseases, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, United States
[d] Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, United States
[e] Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, United States

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ABSTRACT

NK cells, which contribute to immune defense against certain viral infections and neoplasia, are emerging as modifiers of chronic immunologic diseases including transplant rejection and autoimmune diseases. Immunobiology and genetic studies have implicated NK cells as a modifier of Crohn's disease, a condition often treated with thiopurine agents such as 6-mercaptopurine (6-MP). Here, we demonstrate that thiopurines mediate NK cell apoptosis via a caspase 3 and 9 inclusive pathway, and that this process is triggered by thiopurine-mediated inhibition of Rac1. We also show that CD patients in clinical remission maintained on 6-MP have decreased NK cell Rac1 activity, and decreased NK cell numbers in their intestinal biopsies. These observations suggest that thiopurine targeting of NK cells may be a previously unappreciated therapeutic action of these agents in IBD.

1. Introduction

NK cells belong to the innate immune system and are classically understood for their role in host defense responses against certain viruses and cells with malignant potential [1–4]. Key to these roles is the NK cells' receptor repertoire for detection of surface molecules specific to microbial agents or categorically induced by the molecular stress response shared by viral infection and neoplastic states [2,5,6]. Modern studies in NK cell biology have increasingly highlighted that NK cells influence the pathophysiologic processes underlying diverse chronic inflammatory conditions such as transplant rejection [7,8], rheumatoid arthritis [9], diabetes [10], and inflammatory bowel disease (IBD) including ulcerative colitis (UC) and Crohn's disease (CD) [11–15]. The mechanisms by which NK cells affect these inflammatory processes are incompletely understood, but may include direct tissue damage via parenchymal cell cytolysis and production of cytokines inducing T cell or myeloid cell recruitment and activation [16–18].

Intestinal inflammation in CD results from dysregulated actions of adaptive and innate immune cells driven by genetic, environmental, and microbial processes. Although adaptive immune cells are considered the definitive mediators of mucosal inflammation, NK cells have received increasing attention as potential contributors to this immune pathophysiology. Genome-wide studies have associated characteristic NK receptors (killer immunoglobulin-like receptors, KIR) and their cognate HLA alleles with IBD susceptibility [19,20]. At the cellular level, populations of NK cells with cytolytic potential are enriched in colonic lamina propria of individuals with active IBD [11,15], and subsets of NK cells exert inflammatory effect by promoting $CD4^+$ T cell proliferation and CD-relevant Th17 differentiation via production of pro-inflammatory cytokines [16]. Notably, this inflammatory action is dependent on KIR and HLA-dependent genetic programming of NK cells termed 'licensing', and results in distinct subsets of human subpopulations genetically distinct for inflammatory and anti-viral proficiency of their NK cell compartment [16,21–23]. These observations suggest that drugs which affect NK and other innate immune cells may be a significant and underappreciated component of their therapeutic action.

6-mercaptopurine (6-MP) and its precursor drug azathiopurine are immunosuppressive thiopurines frequently used in treatment of hematologic malignancies, chronic inflammatory diseases, and maintenance of graft function following solid organ transplants. 6-MP has been used for over a decade in CD and UC patients to maintain disease

Abbreviations: 6-MP, 6-mercaptopurine; 6-TG, 6-thioguanine; 7-AAD, 7-amino-actinomycin; CD, Crohn's disease; IBD, inflammatory bowel disease; KIR, killer immunoglobulin-like receptor; UC, ulcerative colitis.

* Corresponding author at: Department of Pediatrics, Harbor UCLA Medical Center and Los Angeles Biomedical Institute, Torrance, CA 90502, United States.
E-mail address: syusung@labiomed.org (S. Yusung).

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remission and continues to be a mainstay in therapeutic options for inflammatory bowel diseases [24,25]. Both 6-MP and azathiopurine remain in inactive prodrug form until they are converted by intracellular enzyme hypoxanthine-guanine phosphoribosyltransferase, which is involved in the purine recycling pathway and ubiquitously present in many cell types. Thereafter, the drugs undergo rapid enzymatic conversion to form 6-thioguanine (6-TG) nucleotide, a purine analog and the principal metabolite responsible for immunosuppressive and cytotoxic effects.

Incorporation of 6-TG into replicating DNA structures of immune cells, thereby inhibiting cell proliferation, was long believed to be the therapeutic mechanism of 6-MP. However, studies of the past decade uncovered high affinity and competitive antagonist activity of 6-TG for the small GTPase protein Rac1 [26,27]. Rac1 is involved critical cell functions such as migration, production of soluble signaling mediators, and survival. It alternates between an active and an inactive form in a cycle catalyzed by guanine nucleotide exchange factor (GEF), which catalyzes exchange of GDP and GTP in Rac1, thereby creating the active GTP bound form of Rac1. 6-MP and its metabolite 6-TG interfere with this exchange, leading to inhibition of Rac1 activity [26,28]. In $CD4^+$ T cells, inhibition of Rac1 by 6-MP metabolites induces apoptosis in the presence of co-stimulatory CD28 signal [26]. In macrophages, 6-MP reduces expression of inducible nitric oxide synthase in a Rac1 dependent manner while in intestinal epithelial cells, 6-MP inhibition of Rac1 leads to decreased proliferation and diminished interleukin-8 production [27]. These observations indicate that Rac1 targeting by thiopurines and the consequent effect on cellular function may be an important component of their therapeutic mechanism.

When 6-MP was first adopted as maintenance therapy in IBD, patients on such treatment were reported to have decreased number of peripheral NK cells that correlated with diminished disease activity [11,15,29,30]. However, the mechanism underlying this clinical finding has not been investigated. In the present study, we demonstrate that 6-MP mediates apoptosis of NK cells via a caspase 3 and 9 inclusive pathway, and that this process is triggered by inhibition of Rac1. We also show that CD patients in clinical remission on 6-MP therapy have decreased NK cell Rac1 activity and decreased numbers of NK cells in their intestinal biopsies. These findings provide evidence for the mechanism of NK cell depletion by thiopurines, and for its effect on both peripheral and intestinal NK cell compartments during thiopurine treatment. In view of recent biologic and genetic evidence for roles of NK cells in IBD pathogenesis, these observations suggest that thiopurine targeting of NK cells may be a previously unappreciated therapeutic action of these agents in IBD.

2. Materials and methods

2.1. Clinical samples

Clinical samples were collected according to protocols approved by the institutional review committees in Cedars-Sinai Medical Center (CSMC) and in University of California, Los Angeles (UCLA). Peripheral blood samples from CD patients were collected from patients recruited at CSMC and from healthy donors recruited at UCLA. Intestinal and colon biopsy samples were collected from CD patients and healthy individuals during clinically indicated colonoscopy procedures at UCLA

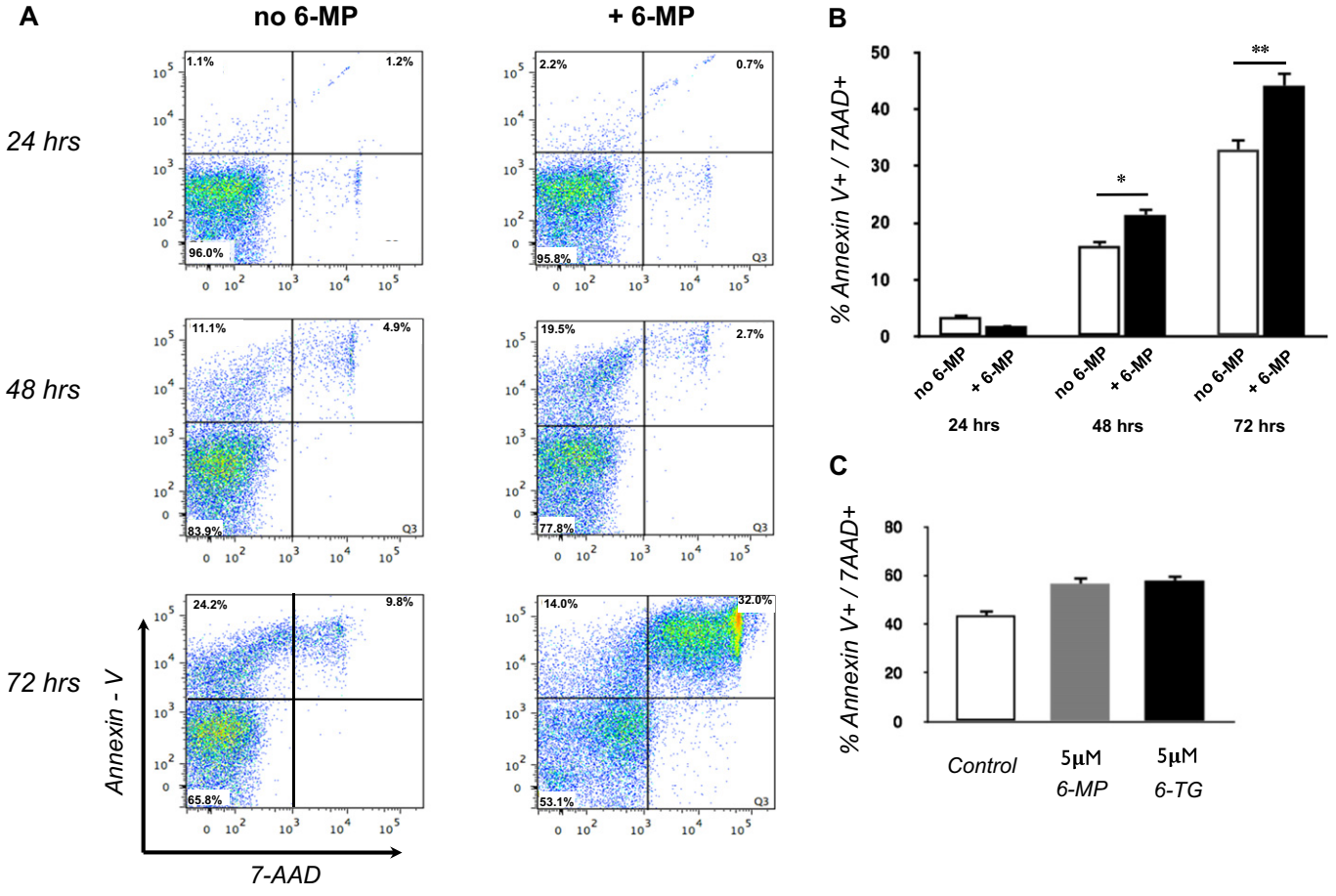

Fig. 1. 6-MP and its metabolite 6-TG induce NK cell apoptosis. (A) Peripheral blood NK cells isolated from healthy individuals were cultured with and without 6-MP and 1 ng mL^{-1} of rIL-2. Representative flow cytometry at 24, 48, and 72 h of culture. (B) Bar plot showing data from 3 experiments tabulated for % Annexin V and 7-AAD positive cells at 24, 48, and 72 h. (Student's *t*-test, two-tailed. $^*p < 0.01$, $^{**}p < 0.005$) (C) Bar plot of Annexin V and 7-AAD positive NK cells at 72 h culture with 5 µmol/L 6-MP or 6-TG. Greater percentage of cells cultured with 6-MP and 6-TG were Annexin V and 7-AAD positive. Plot is representative of separate experiments from 3 healthy individuals. One way ANOVA [$F(2,6) = 25.97$, $p = 0.001$].