# BASIC-ALIMENTARY TRACT

## CD4+NKG2D+ T Cells in Crohn's Disease Mediate Inflammatory and Cytotoxic Responses Through MICA Interactions

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***Background& Aims:*** **Crohn's disease (CD) is an inflammatory bowel disease characterized by uncontrolled immune responses to bacterial flora, with excessive activation of T lymphocytes. MICA is a stress-induced major histocompatibility complex–related molecule expressed on normal intestinal epithelial cells (IECs) and recognized by the NKG2D-activating receptor on $CD8^+$ T cells, $\gamma\delta$ T cells, and natural killer cells. We examined the role of MICA-NKG2D interactions in the activation of T lymphocytes in CD.** ***Methods:*** **MICA expression was analyzed by flow cytometry on IECs isolated from patients with active inflammatory bowel disease and controls. NKG2D expression and function were analyzed on lamina propria and peripheral blood lymphocytes.** ***Results:*** **MICA expression was significantly increased on IECs in CD, with higher expression in macroscopically involved areas. A subset of $CD4^+$ T cells expressing NKG2D was increased in the lamina propria from patients with CD compared with controls and patients with ulcerative colitis. $CD4^+NKG2D^+$ T cells with a Th1 cytokine profile and expressing perforin were increased in the periphery and in the mucosa in CD. $CD4^+NKG2D^+$ T-cell clones were functionally active through MICA-NKG2D interactions, producing interferon-$\gamma$ and killing targets expressing MICA. IECs from patients with CD had the ability to expand this subset in vitro. $CD4^+NKG2D^+$ lamina propria lymphocytes from patients with CD highly expressed interleukin-15R$\alpha$, and interleukin-15 increased NKG2D and DAP10 expression in $CD4^+NKG2D^+$ T-cell clones.** ***Conclusions:*** **These findings highlight the role of MICA-NKG2D in the activation of a unique subset of $CD4^+$ T cells with inflammatory and cytotoxic properties in CD.**

Crohn's disease (CD) is an inflammatory bowel disease (IBD) characterized by uncontrolled immune responses toward the intestinal flora.[1] Breakdown of immune tolerance toward the intestinal contents results in dysregulated and/or constant activation of the immune system. The identification of susceptibility genes for CD, such as *NOD2/CARD15*, suggests an important role of innate immunity in the pathogenesis of uncontrolled inflammation, but it remains clear that adaptive immunity also plays a crucial role in chronic intestinal inflammation. Indeed, a number of experimental systems show that altered regulation of intestinal T-cell function can result in chronic intestinal inflammation.[2,3] In these models, antigenic stimuli from the gut lumen drive the expansion of both effector cells and regulatory T cells, the latter keeping the immune response under control. Chronic intestinal inflammation may be due either to impaired regulatory T-cell activity or excessive effector T-cell function.[3,4] An essential role for $CD4^+$ T cells has been shown in different animal models of experimental colitis.[5] While both Th1 and Th2 cells have been shown able to induce chronic intestinal inflammation in vivo, a Th1 cytokine profile is clearly demonstrated in CD.

Intestinal epithelial cells (IECs) can play a role in the activation of mucosal T cells in IBD. IECs can take up and process antigen and function as antigen-presenting cells.[6] IECs express surface molecules and restriction elements (classic and nonclassic major histocompatibility complex [MHC] molecules) that allow them to interact with unique subsets of T cells.[7,8] In the context of IBD, IECs may drive the expansion of effector T cells with a lack of activation of regulatory T cells.[9,10]

*Abbreviations used in this paper:* CFSE, carboxyfluoroscein succinimidyl ester; ESA, epithelial specific antigen; FITC, fluorescein isothiocyanate; IEC, intestinal epithelial cell; IEL, intraepithelial lymphocyte; IFN, interferon; IL, interleukin; LPL, lamina propria lymphocyte; mAb, monoclonal antibody; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; NK, natural killer; PB, peripheral blood; PBL, peripheral blood lymphocyte; PMA, phorbol myristate acetate; TCR, T-cell receptor; TNF, tumor necrosis factor.


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MICA, an MHC-related class Ib molecule expressed on normal IECs, could be involved in the activation of mucosal lymphocytes. Under normal conditions, expression of MIC molecules (MICA and MICB) is restricted to intestinal and thymic epithelium. This expression can be induced by stress in different epithelial cells and is up-regulated in tumors and upon exposure to intracellular pathogens.[11–13] MICA is a ligand of the NKG2D activating receptor preferentially expressed on $CD8^+$ T cells, γδ T cells, and natural killer (NK) cells.[14–16] MIC molecules function as signals of cellular stress and trigger a range of immune effector mechanisms, including cellular cytotoxicity and cytokine secretion.[17,18] In $CD8^+$ T-cell receptor (TCR) $\alpha\beta^+$ cells, MIC/NKG2D interaction delivers a costimulatory signal that complements TCR-mediated antigen recognition on target cells.[12] NKG2D associates in humans with the DAP10 adaptor protein, allowing transduction of activating signals.[19–21] We previously showed that MICA expression can be markedly increased by exposure to different bacterial strains, including adherent-invasive *Escherichia coli* strains, which have been identified as colonizing the intestinal mucosa of patients with CD.[22,23]

The data obtained in the present study suggest that up-regulation of MICA on IECs in CD may play a role in the activation of a subset of $CD4^+$ effector T cells. Indeed, we identified a subset of $CD4^+$ T cells expressing NKG2D in both mucosa and peripheral blood (PB) of patients with CD. This $CD4^+$ subset exhibits a Th1 cytokine profile and exerts cytotoxic activity against targets expressing MICA. In vitro data suggest a role for IECs and interleukin (IL)-15 in expansion and functional modulation of $CD4^+NKG2D^+$ T cells.

## Materials and Methods

### *Patients With IBD and Controls*

Forty-one patients with moderate to severely active IBD (CD, n = 25; ulcerative colitis [UC], n = 16) were included in this prospective study. Characteristics of the patients are given in Table 1. Peripheral blood lymphocytes (PBLs) were isolated from 34 patients (CD, n = 21; UC, n = 13). IECs and mucosal lymphocytes (lamina propria lymphocytes [LPLs] and intraepithelial lymphocytes [IELs]) were isolated from surgical specimens (n = 15) or endoscopic biopsy specimens (n = 9) of 24 patients (CD, n = 16; UC, n = 8). With surgical specimens of patients with CD, tissues were taken from inflammatory sites and noninflammatory sites when available.

The control group consisted of patients undergoing bowel resection for cancer (n = 4), healthy patients undergoing screening colonoscopy for colorectal cancer (n = 6), or healthy blood donors (n = 16). IECs and mucosal lymphocytes (LPLs and IELs) were isolated from 10 controls, and PBLs were isolated from 20 controls (including 16 healthy blood donors and 4 patients undergoing colonoscopy).

**Table 1.** Clinical Characteristics of Patients With IBD and Controls

| | Patients With CD (n = 25) | Patients With UC (n = 16) | Controls (n = 24) |
|---|---|---|---|
| Age (*y*)[a] | 34 ± 5 | 36 ± 13 | 52 ± 14 |
| Sex (*M/F*) | 10/15 | 11/5 | 11/13 |
| Location of lesions | | | |
| Ileum | 18 | 0 | — |
| Colon | 15 | 16 | — |
| Anus | 6 | 0 | — |
| Active disease | 25 | 16 | — |
| Harvey–Bradshaw[a] | 10 ± 4.2 | — | — |
| Truelove–Witts[b,c] | — | 3 (1–4) | — |
| C-reactive protein (*mg/L*)[b] | 32 (9–333) | 27 (1–105) | Not determined |
| Treatments[d] | | | |
| 5-ASA | 1 | 6 | — |
| Corticosteroids | 4 | 8 | — |
| Antibiotics | 1 | 0 | — |
| Azathioprine/6-mercaptopurine, methotrexate | 8 | 3 | — |

[a]Mean ± SD.
[b]Median (extremes).
[c]Number of criteria.
[d]At study inclusion.

This study was approved by the ethical committee of Hospital Saint-Louis, and all subjects gave written informed consent.

### *IECs and Isolation of Lymphocytes*

Isolation from surgical specimens was performed as described previously.[7] Briefly, surgical specimens were washed extensively with phosphate-buffered saline (PBS). The mucosa was stripped off from the submucosa, minced into small pieces, and placed in 1 mmol/L dithiothreitol for 10 minutes at room temperature. The pieces were washed in PBS and incubated in medium (RPMI 1640) containing 1.5 mmol/L $MgCl_2$ and 1 mmol/L EDTA for 30 minutes at 37°C and vortexed every 5 minutes. The supernatant, containing IECs and IELs, was passed through a nylon filter (Falcon 2360; Becton Dickinson, BD Biosciences, Le Pont de Claix, France). Cells were washed twice in PBS and resuspended in RPMI 1640. For isolation from endoscopic biopsy specimens, the same technique was applied but biopsy specimens were incubated directly in medium (RPMI 1640) containing EDTA, without incubation in dithiothreitol.

LPLs were isolated from the remaining tissue. The tissue was incubated for 1 hour at 37°C in medium containing 1 mg/mL collagenase (Clostridiopeptidase A). The cell suspension was collected, centrifuged, washed, and resuspended in PBS.

Heparinized venous blood was collected from patients or controls, diluted 1:3 with PBS, layered on a Ficoll-