J. Biol. Res. 2011 n.1 - Vol. LXXXIV - Rubbettino - Soveria Mannelli

BT3 molecules ligation enhances the proinflammatory responses of human monocytes and monocyte-derived dendritic cells

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KEY WORDS: BT3; B7 family; monocytes; dendritic cells; Toll-like receptors.

Abstract

BT3, a new family of immunoreceptors, belongs to the extended B7 family. BT3 are expressed on the surface of resting and activated monocytes and monocyte-derived dendritic cells (iDC). We show that BT3 cross-linking, in the absence of other survival factors, provides a survival signal for monocytes. We further analysed the effects of BT3 cross-linking on various proinflammatory responses. Results obtained showed that BT3 engagement is able to modulate production of IL8/CXCL8, IL-1b and IL-12/p70. Moreover, we demonstrated a co-stimulatory effect of BT3 receptors.

Introduction

Priming of T cells is modulated by involvement of specialised cells, secretion of chemotactic cytokines and expression of costimulatory molecules [1].
The BT family of proteins is a subgroup of the Ig superfamily belonging to the extended B7 family [2-4]. In particular, BT3 (BT3.1, BT3.2, and BT3.3) family members share 95% mRNA identity, and are constitutively expressed on the cell surface of antigen-presenting cells, T and B cells [3, 4].
In this paper, we investigated a possible functional role of BT3 receptors by analysing their capability to transduce a functional signal on monocytes and monocyte-derived dendritic cells (iDC). We provide evidence that BT3 transduces survival signals, and enhances proinflammatory responses, measured as secretion of proinflammatory cytokines, such as IL-8/CXCL8, IL-12/p70 and IL-1β, in both cell types.

Materials and methods

Cell cultures. Monocytes and immature dendritic cells (iDC) were selected and obtained as previously described [5].
Flow cytometry and Antibodies. Monocytes and iDCs before and after stimulation were analysed by imunofluorescence flow cytometry to verify their activation state [8].
Apoptosis detection. Apoptosis of monocytes and iDC was evaluated as previously described [5].
Cytokines production. Monocytes and iDC were stimulated with anti-BT3 mAb and TLR4 agonist LPS, or TLR 7/8 agonist R848, or TLR3 agonist poly I:C. IL-8/CXCL8, IL-12/p70 and IL-1β concentration in cell supernatants were measured by ELISA kits (Bender).

Results

BT3 receptors are constitutively expressed on the surface of monocytes and iDCs. Fig. 1A shows BT3 expression on freshly isolated monocytes and iDC. Expression of BT3 was slightly higher on monocytes. Otherwise, BT3 receptors are constitutively expressed on the surface of these cells.
BT3 ligation provides a survival signal for monocytes and iDC in culture. We investigated the ability of BT3 engagement to modify the life span of *ex vivo* monocytes and iDC cultured in the absence of survival factors for 72 hours. Approximately 83% of monocytes treated with anti-BT3 mAb survived in the absence of survival factors for 3 days (52.2-83.3%, $p<0.05$) (Fig 1B, upper row). This effect was slightly lower than that obtained by GM-CSF treatment (average 84.22%, 60.41-85.7%, $p<0.05$). In control cultures (anti-CD19, irrelevant mAb)

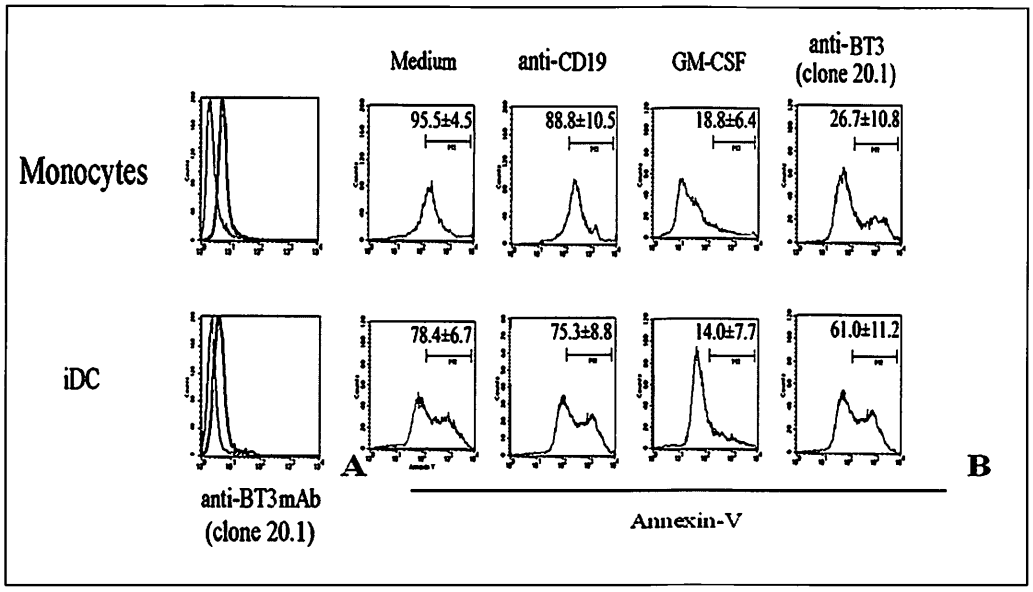

Figure 1. BT3 ligation provides survival signals for monocytes and iDC. Panel A, BT3 receptors are constitutively expressed on the surface of monocytes and iDCs. Panel B, freshly isolated monocytes (upper row) and iDC (lower row) were stimulated with plastic-coated mAb (anti-CD19, and anti-BT3) or 20 ng/ml GM-CSF as indicated. After stimulation for 72 hours, cells were analyzed for Annexin V binding, as a marker of apoptotic cells. Numbers correspond to the percentage of positive cells. Data shown are representative of five independent experiments.

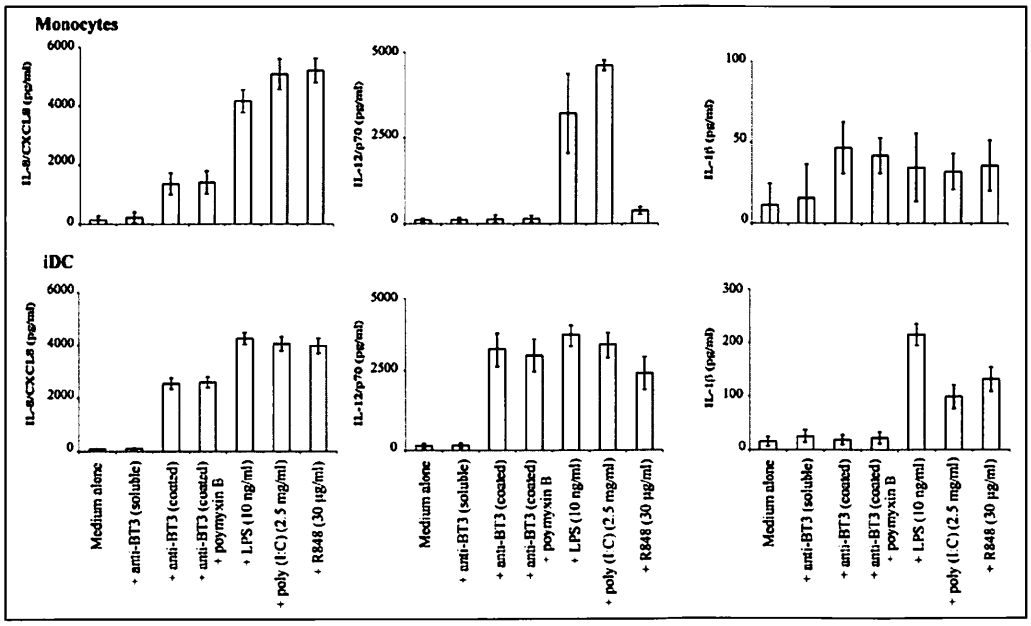

Figure 2. BT3 ligation enhances proinflammatory responses. BT3 ligation and TLR stimulation up-regulate soluble mediator release by monocytes (upper row) and iDC(lower row). Data are mean ± SD of triplicate samples from one representative experiment of three performed with similar results. As control, anti-CD19 irrelevant mAb was added.

survival effect was minimal and the majority of monocytes was apoptotic (about 95% of cells was measured in medium alone and about 89% with anti-CD19 mAb, $p=0.1$). Similar results were obtained by analysing the effect on iDC survival (Fig 1B, lower row). Activation of iDC via BT3 was able to partially inhibit iDC apoptosis during a 72-h culture period (49% of healthy-vital cells versus 86% in the survival GM-CSF treatment, range 54.2-61.0%, $p<0.05$, and 73.4-86.1%, $p<0.05$ respectively). Irrelevant isotype-matched mAb did not alter the naturally occurring apoptosis of DC.

BT3 ligation induces proinflammatory responses. Next, we stimulated monocytes and iDC with anti-BT3, LPS, poly (I:C), and R-848. As shown in Figure 2 (upper panel), we observed an increase in secretion of proinflammatory cytokines (IL-8/CXCL8, IL12/p70, and IL-1β) by monocytes cultured with individual stimuli. Parallel experiments were performed with iDC. The secretion of IL-8/CXCL8 and IL12/p70 was similar in monocytes and iDC, at variance IL-1β production as more marked in iDC than in monocytes (about 3 times).

Finally, we verified that the induction of cytokines secretion mediated by anti-BT3 was mediated by contaminating endotoxin by the addition of polymyxin B.

Discussion

Co-stimulatory molecules play a crucial role to enhance immunoresponse. BT3 is a new co-stimulatory receptor family promoting T-lymphocyte activation [4].

Here, we show that BT3 engagement attenuate apoptosis of monocytes and iDC, providing survival signals. In addition, BT3 induce the secretion of proinflammatory cytokines able to promote the recruitment of immune cells and to stimulate the innate and adaptive response.

As BT3 are stably expressed molecules widely present on immune cells [4], the presence of putative BT3-ligand(s) in the immune environment might play a critical immunoregulatory role in defining where and when BT3 is engaged. BT3 could recognize pathogen components, or be involved in the

recognition of endogenous infection- or stress-induced molecules.

Acknowledgements

This work was supported by a grant from Fondazione CARIGE, Genova, and from Progetti di Ricerca di Ateneo, University of Genova, Italy to D.S.; A.R. is supported by an American-Italian Cancer Foundation Post-Doctoral Research Fellowship. We thank Rosa Piccardo for her secretarial assistance.

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