Cellular Immunology 285 (2013) 55–61

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Human lung fibroblasts increase CD4(+)CD25(+)Foxp3(+) T cells in co-cultured CD4(+) lymphocytes

Enrico Conte *[,1], Elisa Gili [1], Mary Fruciano, Evelina Fagone, Carlo Vancheri

Department of Clinical and MolecularBiomedicine, University of Catania, Catania, Italy

ARTICLE INFO

Article history:
Received 11 April 2013
Accepted 3 September 2013
Available online 12 September 2013

Keywords:
Human lung fibroblasts
T regulatory cells
PGE_2

ABSTRACT

Aim of this study was to evaluate functional modifications induced by human lung fibroblasts in co-cultured CD4(+) T lymphocytes. CD4(+) T cells, resting or stimulated with ionomycin/PMA for 6 h, were co-cultured with fibroblasts isolated from pulmonary biopsies, in contact or separated by a semi-permeable membrane. The expression of CD25, CTLA-4, TGF-β, IFNγ, IL-2, IL-4, IL-10 and Foxp3 was evaluated by flow cytometric analysis. Fibroblasts induced a significant increment in CD25(+) cells in co-cultured activated CD4(+) T lymphocytes separated by a membrane. Moreover, fibroblasts treatment with a COX2 inhibitor abrogated the increment in CD25(+) cells whereas exogenous PGE_2 restored it. The CD25(+) subpopulation was characterized by increased presence of Fox-P3, CTLA-4, IL-10 and TGF-β positive cells while IFN-γ and IL-2 positive cells were diminished. Proliferative response of CD4(+) to the anti CD3/CD28-Abs was abrogated in CD4(+) co-cultured with fibroblasts thus demonstrating a suppressive feature of the expanded CD25(+) subpopulation.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

T cells co-expressing CD4 and interleukin (IL)-2 receptor α-chain (CD25) on the cell surface have been identified as regulatory cells [1]. Forkehead/winged helix transcription factor (Foxp3) has been shown to be necessary and sufficient for the function and development of CD4(+)CD25(+) T regulatory cells [2]. Moreover, a variety of accessory surface molecules, including CTLA-4 and GITR, are involved in the suppressive activity of T-regulatory (Treg) cells [3,4]. Many studies have demonstrated that CD4(+)CD25(+) T cells secrete IL-10 and TGF-β at high levels and the role of these cytokines in differentiation and suppressive activity of T regulatory cells have been reported [5]. Activation of CD4(+) cells in the presence of TGF-β has been shown to increase the expression of CD25 and CTLA-4, therefore this cytokine can induce naïve CD4(+) cells to a regulatory phenotype [6,7]. Together with TGF-β, IL-10 is recognized to play a pivotal role to educate other CD4(+)CD25(-) cells to become cytokine-dependent Treg cells as well as IL-2 is known to increase FoxP3 gene expression in CD4(+) cells, suggesting that these cytokines are fundamental to the development of Treg cells [8].

Recent studies have suggested that Treg cells are defective in many autoimmune disorders [9–11]. A local and systemic defect in Treg levels and activity was recently observed also in patients with idiopathic pulmonary fibrosis (IPF), one of the most common forms of pulmonary fibrosis [10,12]. Moreover, the involvement of T lymphocytes was addressed in IPF [13]. On the other hand, infiltration of activated T cells observed in the lung tissue of patients with IPF has suggested a possible role of these lymphocytes in the pathogenesis of the disease [14]. Particular attention was focused on circulating CD4(+) T cells producing mediators involved in tissue fibrosis [15]. The role of T cells in pulmonary fibrosis seems to be very complex and it might be involved mechanisms of immune tolerance in which Treg cells play a essential control.

Several lines of evidence indicate that human lung fibroblasts play an active role in the modulation of the inflammatory process as well as in the regulation of immune response [16–19]. We have previously shown that human lung fibroblasts inhibit TNF-α and IL-12 production whereas stimulate IL-10 release by monocytes [20,21], effects that are mediated by prostaglandin E2 (PGE_2), likely acting through the inhibition of NF-κB [22]. In addition, we demonstrated that lung fibroblasts are able to affect CD4(+) T cells phenotype and function, modulating not only surface markers known to represent the state of activation of T cells but also some functional properties of T cells [18]. In this study, we investigated the interaction between normal human lung fibroblasts and co-cultured CD4(+) T lymphocytes, in contact or physically separated by a semi-permeable membrane. A significant increment in CD25(+) cells was observed in co-cultured activated CD4(+) T lymphocytes and a role of PGE_2 in this phenomenon was demonstrated. Furthermore, we characterized the induced CD25(+) subpopulation and evaluated its suppressive feature.

* Corresponding author. Address: Dipartimento di Biomedicina Clinica e Molecolare, Sezione di Medicina Respiratoria, Università di Catania, Policlinico Universitario, Via Santa Sofia 78, 95123 Catania, Italy. Fax: +39 095 378 1427.

E-mail address: econte@unict.it (E. Conte).

[1] These authors contributed equally to this work.

0008-8749/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cellimm.2013.09.002

2. Methods

2.1. Fibroblast cultures

Seven primary cell lines of human adult lung fibroblasts were established accordingly to a previously described method [23]. Outgrowths were derived from histologically normal areas of lung specimens from patients undergoing surgical operation for cancer and giving informed consent. Briefly, lung specimens were chopped into pieces of less than 1 mm^3 and washed once with PBS and twice with RPMI-1640 (Gibco, Paisley, UK); containing 10% FCS, penicillin 100 U/ml, streptomycin 100 μg/ml, and fungizone 25 mcμg/ml (supplemented RPMI), eight to ten pieces of washed specimens were then plated in a 100-mm polystyrene dish (Falcon, Becton Dickinson, Lincoln Park, NJ, USA) and overlaid with a coverslip held to the dish with sterile vaseline. Ten milliliters of supplemented RPMI were added and the tissue was incubated at 37 °C with 5% CO_2. The medium was changed weekly. When a monolayer of fibroblast-like cells covered the bottom of the dish, usually 5–6 weeks later, the explant tissue was removed, and the cells were then trypsinized for 10 min, resuspended in 10 ml of supplemented RPMI, and plated in 100-mm tissue culture dishes. Subsequently, cells were split 1:2 at confluence, usually weekly. Aliquots of cells were frozen and stored in liquid nitrogen. In all experiments we used cell lines at a passage earlier than the tenth.

2.2. Isolation of T lymphocytes

Heparinized venous blood, obtained from volunteer healthy donors, was diluted 1:3 with PBS, and 40 ml were then placed on 10 ml of Lymphoprep (Axis-Shield, Oslo, Norway) for centrifugation at 1600 rpm for 35 min at room temperature. Mononuclear cells were collected at the interface, washed three times and resuspended in PBS supplemented with 0.5% bovine serum albumin and 2 mM EDTA. Isolation of human Naïve CD4(+) T lymphocytes separation from mononuclear cells was performed by negative selection of CD4(+) cells using the Regulatory T cells Isolation Kit (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's instructions. The purity of preparations was assessed by FACS analysis to be $\geqslant$95%. For CD25(+) cell characterization cells were directly labeled with CD25 MicroBeads (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) and immunomagnetically isolated by positive selection.

2.3. Lymphocyte–Fibroblast co-cultures

Lymphocytes were incubated in 60 mm polystyrene dish (Falcon, Becton–Dickinson) at a concentration of 4×10^6 cells in 4 ml of supplemented RPMI in the absence or in presence of 1 μg/ml of ionomycin and 10 ng/ml of PMA, plates were then incubated in a humidified atmosphere of 5% CO_2 at 37 °C. After 6 h cells were harvested, washed three times with PBS and counted. Lymphocyte viability was assessed by the trypan blue exclusion method that constantly gave a >90% survival. 1×10^6 unstimulated control or activated lymphocytes were then seeded on top of 0.5×10^6 fibroblasts in 6-well tissue culture plates in a final volume of 2 ml of supplemented RPMI and incubated for 36 h. After the 36 h of co-culture fibroblasts were adherent to the dish and maintained the typical spindle shaped aspect.

Cells were also co-cultured separated by using a cell culture insert (Falcon, Becton Dickinson) with a semi-permeable membrane (0.4 μm pores). In a number of experiments indomethacin (50 μM, Sigma–Aldrich Co., St Louis, MO, USA) was added to fibroblasts during the 36-h incubation time in the absence or in presence of exogenous PGE_2 (1 ng/ml, Cayman Chemical).

2.4. Flow cytometric analysis

Flow cytometric analysis was carried out on T cells isolated and co-cultured with fibroblasts as described before. After 36 h of co-cultured cells were lightly trypsinized, washed and resuspended in PBS with 0.1% BSA. The cells were incubated with PE- or FITC-coupled antibodies: anti-human CD25 monoclonal antibody (mAb) (40 μl/mL, Immunotech, Marseille, France), anti-human CD152 (CTLA-4) mAb (4 μl/ml, Pharmingen-Italia, Milan, Italy) or anti-human TGF-β mAb (1 μl/ml, R&D System-Minneapolis, MN) for 60 min at room temperature. Intracellular staining of cytokines and Foxp3 was performed as previously described [18]. FITC or PE-conjugated anti-cytokine antibodies were used at the following concentrations: anti-human IFN-gamma, anti-human IL-2, anti-human Il-4 and anti-human IL10 (all 2.5 μl/ml, Caltag Laboratories, Burlinghame, CA, USA), anti-human Foxp3 (20 μl/ml, eBIOSCIENCE, ltd, Hatfield, UK). Stainings with PE- or FITC-coupled isotype-matched antibody was performed as control. Fibroblasts and lymphocytes were gated on the basis of forward and side scatter profile. Gating was always restricted on T cells. At least 10,000 forward and side scatter gated events were collected per specimen.

2.5. T lymphocytes proliferation assay

CD4(+) lymphocytes co-cultured with fibroblasts were harvested after 36 h, washed three time with PBS counted and plated at a density of 2.5×10^5 cells in 24 well plates in supplemented RPMI with anti CD3/CD28-Abs (Dynabeads® CD3/CD28; Invitrogen, 2,5 μg/ml) and incubated for 72 h at 37 °C in a 5% CO_2 atmosphere. Thereafter medium was removed and cells were recovered to be counted by FACS analysis with a microsphere kit (Beckman Coulter, Inc., Fullerton, CA, USA).

2.6. Statistical analysis

By using the StatGraph software (StatGraph Inc, Version 2.2, Rockville, MD, USA), statistical comparisons of the number of CD25(+) as well as IL-2, IL-4 IL-10, TGF-β, INF-γ, CTLA-4 and Foxp3 positive cells in all different experimental conditions were performed using a one-way analysis of variance (ANOVA) followed by the Tukey's test, the same tests were used to assess differences among T cells proliferative responses to the anti CD3/CD28-Abs stimulation. A p value less than 0.05 was considered significant.

3. Results and discussion

3.1. Co-culturing activated CD4(+)T lymphocytes with separated lung fibroblasts induced a significant increase in CD25(+) cells

In a previous study, we showed that human normal lung fibroblasts co-cultured with T lymphocytes are able to down-regulate the expression of CD3, CD28, CD69 and LFA-1 as well as TNF-alpha production, some of the main signals identifying T-cell activation and taking active part in the immune response, besides to affect the capacity of T cells to proliferating in response to a non specific stimulus and potentially altering the interaction with antigen presenting cells [18]. In this research we further investigated functional interactions between human lung fibroblasts and CD4(+) T cells, such as the selection/expansion of a CD25(+) subpopulation, analogously to previous observations of other mensenchymal cells [24].

CD4(+)CD25(+) T cells naturally circulating in the blood (nTreg) account for approximately 5% to 10% peripheral CD4(+) T cells and has been shown to regulate the activation of effector T cells in the periphery. As shown in Fig. 1, we observed a mean of 5.67% of

Download English Version:

https://daneshyari.com/en/article/2167172

Download Persian Version:

https://daneshyari.com/article/2167172

Daneshyari.com