Received: 31 August 2023 | Accepted: 2 June 2024

DOI: 10.1111/imm.13824

ORIGINAL ARTICLE

immunology
Insights into immunity in health and disease

IL-10 indirectly modulates functional activity of $CD4^+CD28^{null}$ T-lymphocytes through LFA-3 and HLA class II inhibition

Alejandra García-Torre[1,2,3] | **Eva Bueno-García**[1,2,3] | **Marco A. Moro-García**[2,3,4] | **Rocío López-Martínez**[1,2,3] | **Beatriz Rioseras**[1,2,3] | **Beatriz Díaz-Molina**[2,5] | **José Luis Lambert**[2,5] | **Rebeca Alonso-Arias**[1,2,3]

[1]Immunology Department, Hospital Universitario Central de Asturias, Oviedo, Spain

[2]Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain

[3]Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Oviedo, Spain

[4]Hematology Department, Hospital Universitario Central de Asturias, Oviedo, Spain

[5]Section of Hemodynamics and Interventional Cardiology, Department of Cardiology, Hospital Universitario Central de Asturias, Oviedo, Spain

Correspondence
Rebeca Alonso-Arias, Immunology Department, Hospital Universitario Central de Asturias, Institute of Sanitary Research of Principality of Asturias, 33011 Oviedo, Spain.
Email: rebeca.alonso@sespa.es

Funding information
Gobierno del Principado de Asturias, Grant/Award Number: BP20-030; Instituto de Salud Carlos III, Grant/Award Number: PI17/00714

Abstract

Expansion of $CD4^+CD28^{null}$ T-lymphocytes is common in chronic heart failure (CHF) patients. Its ability to produce high levels of proinflammatory cytokines is probably the key role of these cells in CHF. IL-10 is a candidate for limiting $CD4^+CD28^{null}$ T-lymphocyte responses, whereas tumour necrosis factor (TNF) is the cytokine most closely involved in the loss of CD28 expression. Serum levels of TNF and IL-10 were measured in 65 CHF patients (mean age, 65.2 ± 13.84 years). Patients with an IL-10/TNF ratio ≥1 had significantly lower levels of $CD4^+CD28^{null}$ T-lymphocytes than those with a ratio <1. In vitro, IL-10 reduced the frequency of proliferative $CD4^+CD28^{null}$ T-lymphocytes stimulated with anti-CD3. Pre-treatment with IL-10 before anti-CD3 stimulation was required for the cytokine to inhibit TNF production by $CD4^+CD28^{null}$ T-lymphocytes. In addition to the previously described effect of IL-10 on HLA-DR and ICAM-1 expression, LFA-3 protein and mRNA levels were reduced in the presence of the cytokine in monocytes. IL-10 inhibition on $CD4^+CD28^{null}$ T-lymphocytes may be mediated by a reduction in HLA class II and LFA-3 expression because blocking interactions with these costimulators has similar effects to those of IL-10 treatment. Moreover, costimulation through CD2/LFA-3 interaction is enough to induce proliferation and cytokine production in $CD4^+CD28^{null}$ T-lymphocytes.

Abbreviations: APC, allophycocyanin; CD, cluster of differentiation; CFSE, carboxyfluorescein succinimidyl ester; CHF, chronic heart failure; EDTA, ethylenediaminetetraacetic acid; FITC, fluorescein isothiocyanate; GmbH, Gesellschaft mit beschränkter Haftung; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HLA, human leukocyte antigen; ICAM-1, intercellular adhesion molecule 1; IFN-γ, interferon-γ; IL, interleukin; IQR, interquartile range; LFA-3, lymphocyte function-associated antigen 3; mAb, monoclonal antibody; MFI, median fluorescence intensity; mRNA, messenger RNA; NK, natural killer; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PE, phycoerythrin; PerCP, peridinin chlorophyll-A protein; RPMI, Roswell Park Memorial Institute medium; TCR, T cell receptor; TNF, tumour necrosis factor.

KEYWORDS
chronic heart failure, immunosenescence, T-lymphocyte differentiation

INTRODUCTION

Throughout their differentiation, T-lymphocytes undergo a series of phenotypic and functional changes, one of the most relevant of which is the loss of CD28 expression. The interaction between CD28 and B7 in antigen-presenting cells is responsible for the second signal in the activation process and is indispensable for avoiding T-lymphocyte anergy in the initial stages of its differentiation. Highly differentiated $CD4^+CD28^{null}$ T-lymphocytes are characterised by their low activation threshold and the enhancement of certain properties. These include the expression of several receptors commonly associated with natural killer (NK) cells, the secretion of large amounts of tumour necrosis factor (TNF) and IFN-γ, and the expression of perforin and granzyme B, which gives cells a cytotoxic capability [1–3]. Inflammatory environments may contribute to the expansion of $CD4^+CD28^{null}$ T-lymphocytes because TNF is responsible for a stable loss of CD28 expression in T-lymphocytes [4]. Expansion of $CD4^+CD28^{null}$ T-lymphocytes is common in individuals who experience chronic inflammation, such as elderly people and patients with chronic infections and autoimmune diseases [5–8]. Chronic heart failure (CHF) is a pathology characterised by the non-specific activation of the immune system, which results in an exaggerated production of proinflammatory cytokines. In CHF patients, increased circulating levels of $CD4^+CD28^{null}$ T-lymphocytes have been associated with disease severity and are recognised as an independent predictor of mortality [9, 10].

Little is known about the regulatory mechanisms directed towards limiting $CD4^+CD28^{null}$ T-lymphocyte responses in a way that keeps them from being highly elevated and harmful to the body. One possible candidate is IL-10, a cytokine whose immunosuppressive functions reduce the tissue damage caused by excess and uncontrolled inflammatory effector responses, especially during the resolution phase of infection and inflammation. IL-10 is produced by almost all subsets of leukocytes, but T-lymphocyte subsets are major cellular sources, and this cytokine inhibits proinflammatory responses by acting on most of the innate and adaptive immune cells [11–13]. IL-10 mainly regulates T-lymphocyte activity by inhibiting antigen presentation of monocytes/macrophages, thereby reducing expression of HLA class II, costimulatory, and adhesion molecules, and the production of cytokines [14–16].

The modulation of activity within this subset has not been studied in depth, and the mechanisms involved in this process have not been defined, so for these reasons our study set out to analyse whether IL-10 can also have an inhibitory effect on the functional capacity of $CD4^+CD28^{null}$ T-lymphocytes.

METHODS

Study subjects

Blood samples were obtained from 65 CHF patients (17 females) who were followed up at the Cardiology Department of Hospital Universitario Central de Asturias (Oviedo, Spain). Six healthy volunteers from the Health Centre of El Cristo (Oviedo, Spain), matched by age and sex with the patients, were recruited to the study (2 females; age: 65.3 ± 11.2). Informed written consent was given prior to the inclusion of subjects in the study. Ethical approval for this study was obtained from the Regional Ethics Committee for Clinical Research (Comité de Ética de la Investigación del Principado de Asturias, number 82/17). The study was conducted according to the guidelines of the Declaration of Helsinki. Patients with stable CHF were recruited, i.e., those without decompensation for a minimum of 1 month prior to their inclusion, regardless of their aetiology, and with affected systolic function (ejection fraction <40%). The various experiments analysing the effects of IL-10 on $CD4^+CD28^{null}$ T-lymphocytes were performed on individuals from this subset with a lymphocyte percentage of ≥10%.

Cytokine quantification

TNF and IL-10 levels were measured in the sera of the participating CHF patients using X-Map technology with a Milliplex map kit (Sigma Aldrich, Saint Louis, MO, USA), following the manufacturer's specifications.

Quantification and characterisation of $CD4^+CD28^{null}$ T-lymphocytes

The percentage of $CD4^+CD28^{null}$ T-lymphocytes was determined in peripheral blood samples from the participants in the study by staining them with anti-CD3 (FITC), anti-CD28 (PE) (eBioscience, San Diego, CA, USA), anti-CD8

(PerCP) and anti-CD4 (APC) (BioLegend, San Diego, CA, USA). One hundred microliters of whole blood were stained with the combination of labelled monoclonal antibodies (mAbs) for 20 min at room temperature. Red blood cells were lysed with FACS Lysing Solution (BD Biosciences, San Jose, CA, USA), washed in PBS, and acquired and analysed in a BD Accuri C6 (BD Biosciences). Appropriate isotype-control mAbs were used for marker settings. The gating strategy of $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes is shown in Supplementary Figure 1a.

Phenotypic characteristics of $CD4^+CD28^{null}$ T subset were determined by flow cytometry. Cells were stained with anti-CX3CR1 (FITC) (BioLegend), anti-NKG2D (PE) (BD Bioscience), anti-granzyme B (FITC) anti-perforin (PE), anti-CD3 PerCP, and anti-CD28-APC (BD Bioscience) and anti-CD4-ECD (Beckman Coulter Life Science, Indianapolis, IN, USA). The frequencies of cells with intracytoplasmic stores of granzyme B and perforin were counted. Cells were surface-stained for 20 min at room temperature, lysed and fixed with FACS Lysing Solution, permeabilised with BD FACS Permeabilising Solution 2 (Perm II) (BD Bioscience), and stained with anti-granzyme B-FITC or anti-perforin-FITC for 30 min at room temperature. Cells were washed and resuspended in PBS until they were acquired in a Navios flow cytometer and analysed with Kaluza software (Beckman Coulter Life Science).

Isolation and cell culture

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood anticoagulated with EDTA by centrifugation on Ficoll–Hypaque gradients (Lymphoprep; Nycomed, Oslo, Norway). Monocytes were isolated from PBMCs by positive selection in columns with CD14 MicroBeads (Miltenyi Biotec, GmbH, Bergisch Gladbach, Germany). $CD4^+$ T-lymphocytes were enriched with Human $CD4^+$ Enrichment Cocktail (STEMCELL Technologies, Grenoble, France), following the manufacturer's instructions. In both cases, the purity was >95%.

Cultures were grown in an RPMI 1640 medium supplemented with 10% FCS (ICN Flow, Costa Mesa, CA, USA) and antibiotics. Cells were incubated at 37°C in an atmosphere of 5% carbon dioxide. Cytokines and activating or blocking antibodies were added to the cultures at the concentrations and in the combinations required by the experimental conditions.

Stimulation for cytokine assays

PBMCs (2×10^6 cells/mL) were cultured in medium or in the presence of IL-10 (100 ng/mL) (BioLegend). Soluble anti-CD3 (1 μg/mL; eBioscience, San Diego, CA, USA) was added to the culture at the beginning or after 18 or 36 h of preculture, depending on the experiment. In the blocking experiments, anti-HLA class II (2 μg/mL), anti-LFA-1 (10 μg/mL), anti-LFA-3 (2 μg/mL), anti-OX40 (2.5 μg/mL) (R&D Systems, McKinley Place NE, Minneapolis, MN, USA) and anti-4-1BB (2 μg/mL) (BioLegend) antibodies were also added to the cultures before they were stimulated with anti-CD3.

Isolated monocytes and $CD4^+$ T-lymphocytes were independently precultured in medium alone, or in the presence of IL-10, for 18 h. Cells were then washed, mixed in culture, and stimulated for 18 h with anti-CD3 before intracytoplasmic levels of TNF were quantified.

In other experiments, isolated $CD4^+$ T-lymphocytes were cultured in the presence or absence of IL-10 for 18 h and then stimulated for 18 h with T Cell Activation/Expansion beads (ratio 1:2) (Miltenyi Biotec) that had previously been covered with anti-CD3 and anti-CD2 antibodies, following the manufacturer's instructions.

For the intracytoplasmic staining, cells were treated during the final 2 h of stimulation with the secretion-inhibitor brefeldin A (10 mg/mL) (Calbiochem, Darmstadt, Germany). Cells were surface-stained with anti-CD28 (BV) (BioLegend), anti-CD3 (PerCP) (BD Biosciences) and anti-CD4 (APC) antibodies, then lysed and fixed with FACS lysing solution, permeabilised with BD FACS Permeabilising Solution 2 (Perm II) (BD Bioscience), and stained with anti-TNF (PE) (BD Bioscience) for 30 min at room temperature. Cells were washed and resuspended in PBS until they were acquired in a Navios flow cytometer and analysed with Kaluza software (Beckman Coulter Life Science).

Surface expression of costimulatory molecules

The expression of several costimulatory ligands on the monocyte surface in response to IL-10 was studied. Isolated monocytes were cultured for 24 and 48 h in the medium alone, or in the presence of IL-10 (100 ng/mL), and then stained with anti-HLA class II (PECy7), anti-ICAM-1 (PE), anti-LFA-3 (PE), anti-OX40L (PE) and 4-1BBL (PE) (eBioscience, San Diego, CA, USA), as described above. Cells were acquired in a Navios flow cytometer and analysed using Kaluza software.

mRNA expression

Purified monocytes were cultured in the presence of IL-10 (100 ng/mL) for 18 h. mRNA was extracted using a Total RNA Isolation kit (Macherey-Nagel GmbH &

Co. KG, Düren, Germany), and reverse transcription of mRNA was carried out with the iScript cDNA Synthesis Kit (Bio-Rad, Life Science Research Group, Hercules, CA, USA), following the manufacturer's instructions.

LFA-3 mRNA expression was quantified using TaqMan-Gene-Expression CD58 (Hs00156385_m1) (Thermo Fisher Scientific, MA, USA) and an Applied Biosystems™ StepOne™ Real-Time PCR System (Thermo Fisher). Reactions without cDNA were always included as negative controls. Experimental samples were run in duplicate, and the average value of the replicates was taken as the sample result.

Proliferation assay

PBMCs were resuspended in PBS at a final concentration of 1×10^6 cells/mL, incubated with 0.5 μM CFSE (Invitrogen, Paisley, Scotland, UK) for 10 min at 37°C, and then washed twice with an RPMI 1640 medium containing 2×10^3 M L-glutamine and HEPES. Resting cells and cells stimulated with anti-CD3 (1 μg/mL) were cultured at 2×10^6 cells/mL in the presence or absence of IL-10 (100 μg/mL) for 7 days. The proliferative responses of $CD4^+CD28^{null}$ T-lymphocytes were analysed in a Navios flow cytometer (Beckman Coulter Life Science) after staining with anti-CD28 (PE), anti-CD8 (PerCP), and anti-CD4 (APC).

To quantify the proportion of Ki-67^+ cells, PBMCs were cultured with or without IL-10 and stimulated with anti-CD3 for 4 days before intranuclear staining. In addition, blocking of ligand-receptor interactions was performed as described above. Isolated $CD4^+$ T-lymphocytes were cultured with or without IL-10 and stimulated with anti-CD3 + anti-CD2 coated beads. Anti-LFA-3 was added to the cultures to block possible contamination with non-$CD4^+$ T-lymphocytes.

After 4 days, cells were stained with anti-CD28 (PE), anti-CD8 (PerCP) and anti- CD4 (APC) for 20 min at 4°C. Cells were then fixed and permeabilised with Fixation/Permeabilisation Solution (Invitrogen), prepared according to the manufacturer's instructions, and stained with anti-Ki-67 (PECy7) before being acquired by Navios flow cytometry and analysed with Kaluza software (Beckman Coulter Life Science).

Statistical analysis

The results are expressed as the median and interquartile range (IQR), or the mean and standard deviation in some graphs. Groups were compared using the Mann–Whitney *U* test, since the data were not normally distributed. Pair-wise comparisons were made using the Wilcoxon non-parametric method when data were not normally distributed, or Student's *t* test for paired samples for normally distributed variables. Comparisons of three or more groups were made using the non-parametric Friedman test and the Wilcoxon non-parametric method. Analyses were performed using PASW Statistics 17.0 software (IBM SPSS, NY, USA). Values of $p < 0.05$ were considered statistically significant ($^*p < 0.05$, $^{**}p < 0.01$).

RESULTS

IL-10 production in CHF patients

To analyse whether IL-10 may modulate $CD4^+CD28^{null}$ T-lymphocyte levels, and because TNF is known to be the main cytokine involved in loss of expression of the CD28 molecule, we compared percentages and absolute counts of $CD4^+CD28^{null}$ T-lymphocytes in CHF patients relative to their IL-10/TNF ratio. Table 1 shows the characteristics of the patients according to the IL-10/TNF ratio. Those with a lower ratio (<1; $n = 45$) had significantly higher levels of $CD4^+CD28^{null}$ T-lymphocytes than those with an IL-10/TNF ratio ≥1 ($n = 20$), in terms of percentages and absolute counts (Figure 1). The majority of $CD4^+CD28^{null}$ T-cells expressed CX3CR1 and NKG2D, and had intracytoplasmic stores of granzyme B and perforin (Supplementary Figure 1b).

Effect of IL-10 on $CD4^+CD28^{null}$ proliferation and cytokine production

IL-10 may play a role in $CD4^+CD28^{null}$ T-lymphocyte subset expansion, although it depends on TNF levels. We compared the effect of IL-10 on the proliferation induced by anti-CD3 in $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes. PBMCs, but not isolated $CD4^+$ T-lymphocytes, were cultured because there was no current clear definition of $CD4^+CD28^{null}$ T-lymphocyte costimulatory requirements. A high frequency of cycling cells was found in both subsets of $CD4^+$ T-lymphocytes stimulated with anti-CD3 for 4 days (Figure 2a,c). However the reduction of the percentage of Ki-67^+ $CD4^+$ lymphocytes in the presence of IL-10 was more important in the $CD4^+CD28^{null}$ compartment than in $CD4^+CD28^+$ T-lymphocytes (Figure 2d). Similarly, the proliferative response to anti-CD3 stimulation for 7 days, measured with CFSE staining (Figure 2b,c), demonstrated that $CD4^+CD28^{null}$ T-lymphocytes proliferated more slowly

TABLE 1 Donor features in relation to IL-10/TNF ratio.

	IL-10/TNF <1 ($n = 45$)	IL-10/TNF ≥1 ($n = 20$)	p (between-group)
Demographic and clinical data			
Age ± SD	64.1 ± 12.5	68.2 ± 16.6	NS
Male (%)	32 (71.1)	16 (80)	NS
% LVEF (IQR)	40.0 (53.0)	35.0 (43.0)	NS
NYHA functional class			
I	4 (8.9)	4 (20)	NS
II	19 (42.2)	8 (40.0)	NS
III	20 (44.4)	5 (25)	NS
Missing	2 (4.4)	3 (15)	NS
CHF aetiology			
Coronary artery disease (%)	18 (40.0)	9 (45.0)	NS
Hypertensive heart disease (%)	27 (60.0)	11 (55.0)	NS
CRP, mg/dL (IQR)	1.4 (24.9)	1.5 (21.1)	NS
Cholesterol ± SD (mg/dL)	164.2 ± 30.7	163.4 ± 39.9	NS
Haematological variables (median ± IQR)			
WBCs (10^3/μL)	8.1 ± 1.9	8.3 ± 3.1	NS
Neutrophils (10^3/μL)	5.6 ± 1.8	6.2 ± 3.2	NS
Neutrophils (%)	67.6 ± 9.0	71.9 ± 12.4	NS
Monocytes (10^3/μL)	0.6 ± 0.2	0.6 ± 0.3	NS
Monocytes (%)	8.1 ± 2.4	7.9 ± 2.9	NS
Lymphocytes (10^3/μL)	1.7 ± 0.7	1.4 ± 0.8	NS
Lymphocytes (%)	21.1 ± 8.1	18.2 ± 10.3	NS

Abbreviations: CRP, C-reactive protein; IQR, interquartile range; LVEF, left ventricular ejection fraction; NS, not significant; NYHA, New York Heart Association; SD, standard deviation; WBCs, white blood cells.

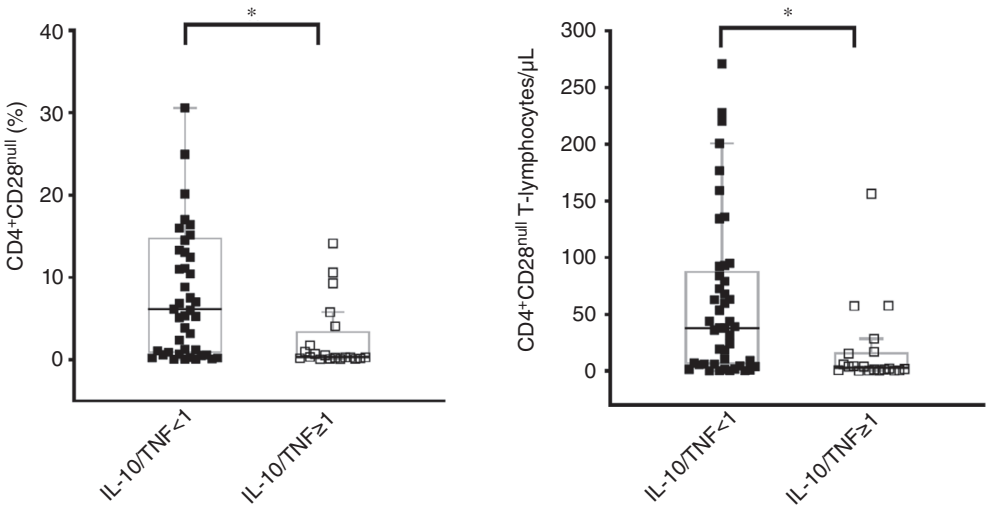

FIGURE 1 Percentages and absolute counts of $CD4^+CD28^{null}$ T-lymphocytes according to IL-10 and TNF levels. CHF patients were classified into two groups with IL-10/TNF ratios of <1 ($n = 45$) or ≥1 ($n = 20$). The Mann–Whitney U test was used to compare the two groups, levels of significance for which are shown in the panels. *$p < 0.05$.

than $CD4^+CD28^+$ T-lymphocytes in the presence of IL-10 (Figure 2d).

To evaluate IL-10's ability to limit $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes response, we studied its effect on TNF production induced by anti-CD3 stimulation. Treatment with IL-10 simultaneously with anti-CD3 stimulation for 18 h had no effect on cytokine production (Figure 3a; t = 0h). However, when cells were precultured for both 18 h and 36 h in medium, or medium containing IL-10, and then stimulated with

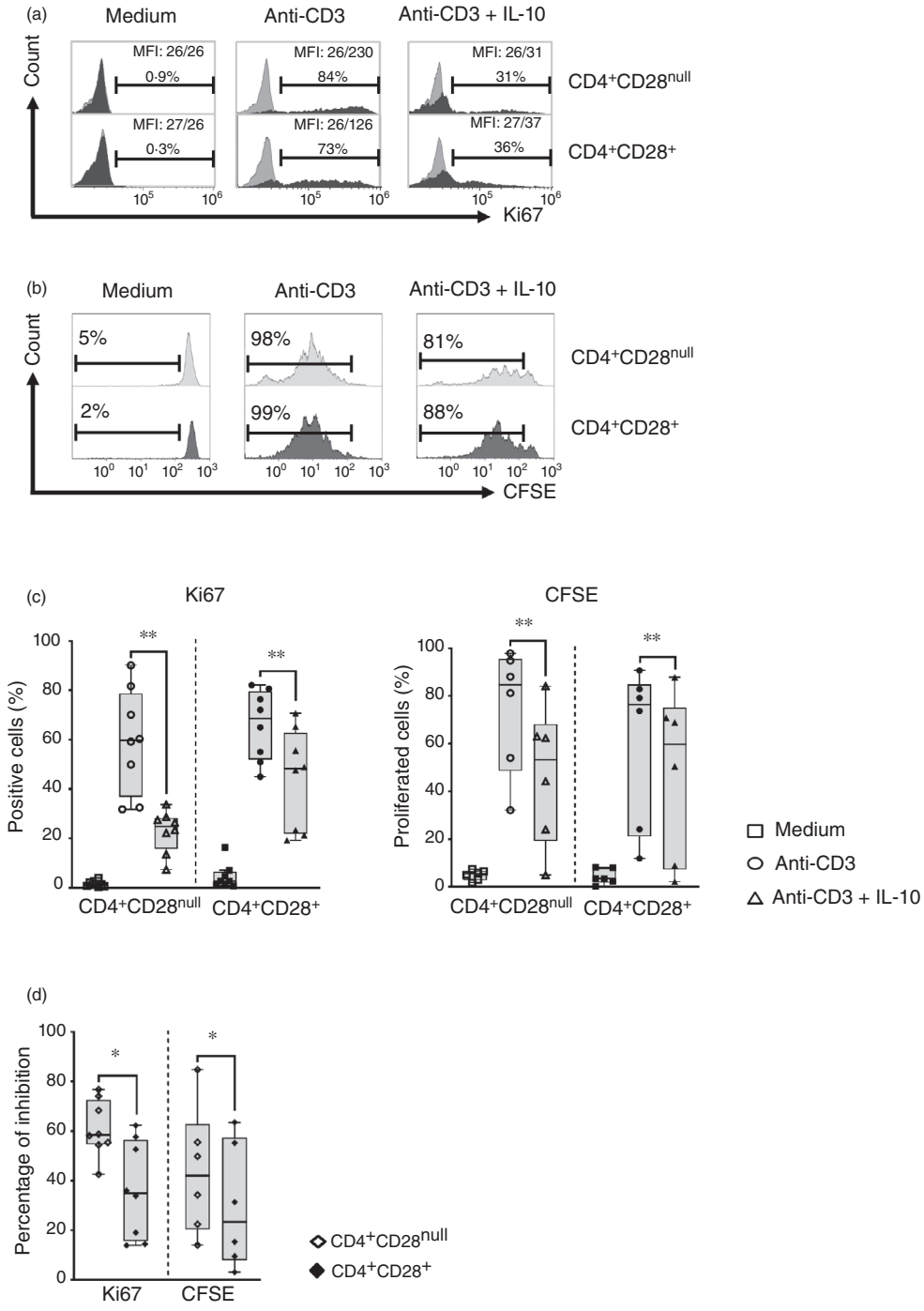

FIGURE 2 Effect of IL-10 on $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocyte proliferation. (a) PBMCs were cultured in medium or stimulated with anti-CD3 (1 μg/mL) in the presence or absence of IL-10 (100 ng/mL) for 4 days. The responder cells in the $CD4^+CD28^{null}$ T-lymphocyte subset were analysed with intranuclear Ki-67 staining ($n = 8$). Percentages of Ki-67-positive cells and MFI (Isotype control/Ki-67 staining) in the $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocyte subsets in this representative experiment are indicated in the histograms (Isotype control: grey; Ki-67 staining: black). (b) PBMCs were labelled with CFSE (0.5 μM) and cultured in medium or stimulated with anti-CD3 (1 μg/mL) in the presence or absence of IL-10 (100 ng/mL) for 7 days ($n = 6$). Histograms show percentages of dividing cells at 7 days of culture in this representative experiment. (c) Box-and-whisker plots represent the percentages of $KI67^+$ and proliferated cells in medium (squares), when stimulated with anti-CD3 (circles) or stimulated with anti-CD3 in the presence of IL-10 (triangles) in gated $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes. Individual values are shown in scatter plots. (d) Box-and-whisker plots show the percentages of inhibition of Ki-67^+ and proliferated cells in gated $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes. Individual values are shown as scatter plots. Paired-samples *t* tests were used to compare groups. $^*p < 0.05$; $^{**}p < 0.01$.

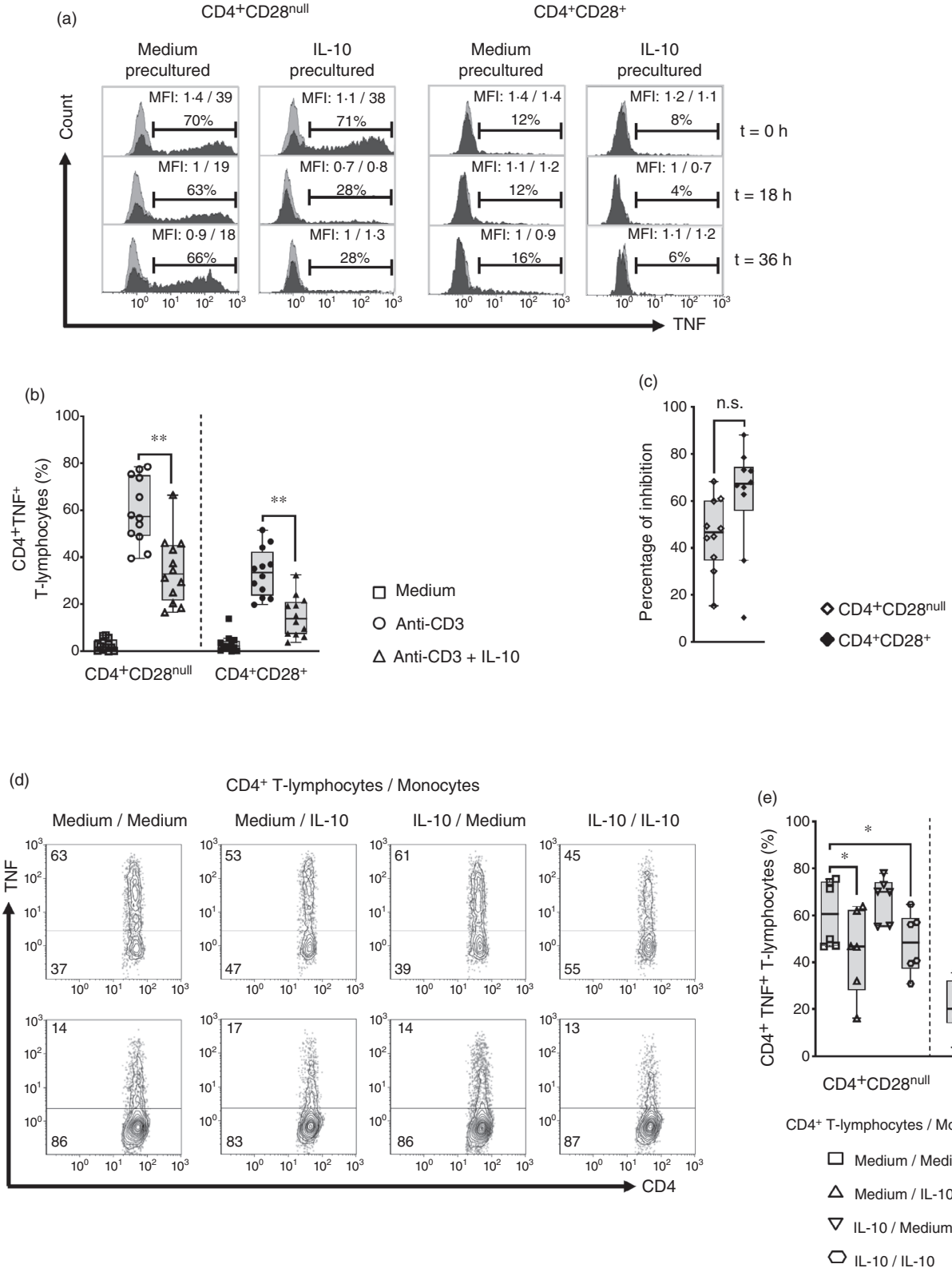

FIGURE 3 Legend on next page.

anti-CD3, IL-10 treatment induced significant inhibition of TNF production in $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes (Figure 3a,b; t = 18 h and t = 36 h). Nevertheless, we found significant differences between the two subsets of $CD4^+$ T-lymphocytes in the percentage reduction of TNF production in the presence of IL-10 (Figure 3c).

To identify whether IL-10 acts directly on $CD4^+CD28^{null}$ T-lymphocytes' ability to respond, or whether it modulates the expression of some costimulatory molecules in antigen-presenting cells, $CD4^+$ T-lymphocytes and monocytes were isolated and independently precultured in medium alone, or in the presence of IL-10, for 24 h. Cytokine levels were reduced in $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes only in the cultures with IL-10-treated monocytes (Figure 3d), but these differences were only significant in the $CD4^+CD28^{null}$ compartment (Figure 3e).

The effect of IL-10 on $CD4^+CD28^{null}$ T-lymphocytes was not specific to cells from CHF patients since similar results were obtained when cells from age- and sex-matched healthy donors were analysed (Supplementary Figure 2).

Relevance of costimulation of $CD4^+CD28^{null}$ T-lymphocyte to their functional activity

To evaluate molecules potentially involved in the modulatory effects of IL-10, we studied the expression of HLA class II, ICAM-1, LFA-3, OX40L and 4-1BBL at the monocyte surface in response to the cytokine. As expected, IL-10 treatment reduced not only HLA class II and ICAM-1 expression, but also levels of LFA-3 (MFI) (Figure 4a and Supplementary Figure 3). In fact, quantification of LFA-3 mRNA expression by RT-PCR in purified monocytes also showed a reduction following 18 h of culture with IL-10 (Figure 4b). OX40L or 4-1BBL showed neither basal expression nor IL-10 modulation in monocytes (Figure 4a; n.s. = not significant). Based on the lack of inhibition caused by IL-10 pretreatment on CD4, this cytokine showed no effect on the studied costimulatory receptors LFA-1, CD2, OX40, and 4-1BB in $CD4^+CD28^{null}$ T-lymphocytes at basal expression or after induction by anti-CD3 activation (Figure 4c and Supplementary Figure 3).

On the other hand, to analyse the relevance of HLA class II/CD4, CD54/LFA-1, and LFA-3/CD2 interactions in the $CD4^+CD28^{null}$ T-lymphocyte response to anti-CD3 activation we blocked the ligand-receptor interaction with specific mAbs. Proliferation in response to anti-CD3 of $CD4^+CD28^{null}$ T-lymphocytes was diminished, but only when anti-HLA class II (the most effective) or anti-LFA-3 blocking antibodies were added to the culture. Similar results were found with simultaneous blocking of both costimulators in the absence of IL-10. These were conditions in which proliferation was almost completely suppressed (Figure 4d,e). Regarding the effect of costimulation on cytokine production, inhibition was noted when the HLA class II/CD4 interaction was prevented, but to a lesser extent than when the CD2/LFA-3 interaction was blocked with anti-LFA-3 antibodies. The lowest levels of TNF were produced when the two interactions were simultaneously blocked, which produced an additive inhibition (Figure 4d,e). However, no effects were found on proliferation and cytokine production when ICAM-1/LFA-1, OX40/OX40L or 4-1BB/4-1BBL interactions were blocked (Supplementary Figure 4).

FIGURE 3 Effect of IL-10 on TNF production by $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes. (a) PBMCs were cultured in medium or in the presence of IL-10 (100 ng/mL). Anti-CD3 was added to the culture at the same time as IL-10 ($t = 0$ h) or $t = 18$ h or $t = 36$ h later ($n = 12$). Percentages of TNF-positive cells and MFI (Isotype control/TNF staining) in the $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocyte in this representative experiment are shown in the histograms (Isotype control: grey; Ki-67 staining: black). (b) Box-and-whisker plots represent the percentages of TNF-positive $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes cultured in medium (squares), when stimulated with anti-CD3 (circles) and stimulated with anti-CD3 in the presence of IL-10 (triangles). (c) Box-and-whisker plots represent the percentages of inhibition of TNF production in $CD4^+CD28^{null}$ (open diamonds) and $CD4^+CD28^+$ T-lymphocytes (filled diamonds). (d) $CD4^+$ T-lymphocytes and monocytes were independently isolated and precultured in medium alone, or in the presence of IL-10 (100 ng/mL), for 18 h ($n = 6$). Cells were then washed, mixed in culture, and stimulated for 6 h with anti-CD3 (1 μg/mL) before quantifying intracytoplasmic levels of TNF. Percentages of TNF-positive cells in the $CD4^+CD28^{null}$ (above) and $CD4^+CD28^+$ (below) T-lymphocyte subsets in this representative experiment are indicated in the dot plots. (e) Box-and-whisker plots represent the percentage of TNF-positive $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocytes when $CD4^+$ T-lymphocytes and monocytes were precultured independently in medium (squares), when $CD4^+$ T-lymphocytes were precultured in medium and monocytes with IL-10 (triangles), when $CD4^+$ T-lymphocytes were precultured with IL-10 and monocytes in medium (inverted triangles), and when both subsets were precultured with IL-10 (hexagon). In all cases, individual values are shown in the scatter plots. Statistics: paired-samples *t* tests (b and c); Friedman and Wilcoxon tests (e) (all comparisons with respect to 'medium/medium' condition are represented). $^*p < 0.05$; $^{**}p < 0.01$, n.s. = not significant.

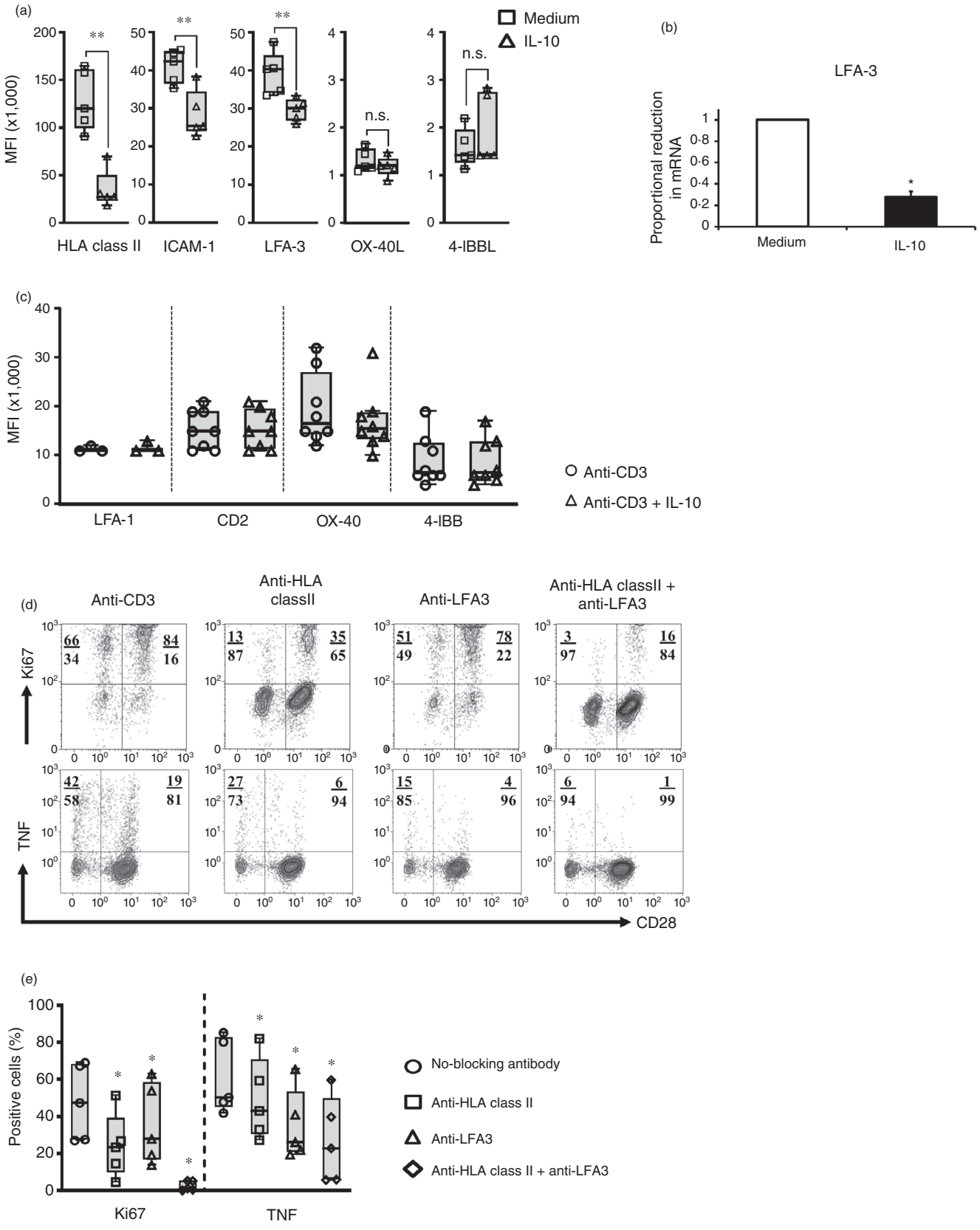

FIGURE 4 Legend on next page.

Costimulation through the CD2 receptor is sufficient to induce $CD4^+CD28^{null}$ T-lymphocyte activation

To characterise $CD4^+CD28^{null}$ T-lymphocyte costimulation through CD2, isolated $CD4^+$ T-lymphocytes were stimulated for 18 h with anti-CD3-coated beads or anti-CD3 + anti-CD2-coated beads. The proportion of Ki-67^+ cells increased in response to CD2 costimulation after 4 days of culture, as did the production of TNF (Figure 5a). Anti-CD3-coated beads induced a low but significant proportion of Ki-67^+ cells and a low level of production of TNF, while addition of anti-CD2 stimulation was enough to activate $CD4^+CD28^{null}$ T-lymphocytes (Figure 5b).

DISCUSSION

The functional properties of $CD4^+CD28^{null}$ T-lymphocytes have been widely studied and described in a variety of pathological situations and in the context of ageing. However, little is known about the mechanisms involved in downregulating the enhanced activity of these cells. In this study, we have demonstrated the inhibitory effects of IL-10 on the proliferation and cytokine production of $CD4^+CD28^{null}$ T-lymphocytes. These effects are at least partially mediated by reductions in HLA class II and LFA-3 expression on the monocyte surface, and our results demonstrate, for the first time, the relevance of the role of LFA-3 in costimulating $CD4^+CD28^{null}$ T-lymphocytes.

CHF is a pathology characterised by non-specific activation of the immune system, resulting in an strikingly high level of production of proinflammatory cytokines, such as TNF, IL-6, IL-1, and IL-18, which is positively correlated with disease severity [17–21]. Anti-inflammatory cytokines, such as IL-10, downregulate the production of several inflammatory cytokines from macrophages and other cells [22,23], and improved heart function has been suggested to be associated with an increased IL-10/TNF ratio [24]. The importance of T-lymphocytes in cardiac dysfunction in the pathogenesis of inflammatory heart diseases is indisputable. Specifically, circulating levels of $CD4^+CD28^{null}$ T-lymphocytes have been associated with CHF severity and identified as an independent predictor of patient mortality [9,25]. We found significantly higher levels of $CD4^+CD28^{null}$ T-lymphocytes in patients with a low IL-10/TNF ratio. It has long been known that TNF induces a reduction in CD28 expression [4,26], but we also found that IL-10 inhibits $CD4^+CD28^{null}$ T-lymphocyte proliferation. This may explain the reduced levels of the subset of patients with an IL-10/TNF ratio >1 because not only CD28 loss, but also subset expansion, may be responsible for the higher counts of these cells in CHF patients. The ability to produce high levels of proinflammatory cytokines is probably the key to the role played by $CD28^{null}$ cells in heart failure, since this contributes to the general inflammatory status of these patients. $CD4^+CD28^{null}$ T-lymphocytes are characterised by their low activation threshold, which could play a part in the predisposition to breaking self-tolerance [27,28], and to enhancing chronic immune responses, thereby contributing to the development of chronic inflammatory diseases. Some studies have described hitherto unknown phenotypic and functional properties of $CD4^+CD28^{null}$, such as the acquisition of the expression of several receptors commonly associated with NK cells, new migration properties or secretion of large amounts of proinflammatory cytokines [2,29]. In contrast, little has so far been reported about the regulatory mechanisms directed towards limiting these responses in a way that keeps them from being so strongly expressed that it harms the body. IL-10 could be a candidate because it inhibits proinflammatory responses acting on most of the innate and adaptive immunity cells. Direct effects of IL-10 in T-lymphocytes have been reported, although we found none in

FIGURE 4 Effect of IL-10 on expression of molecular costimulatory molecules. (a) Box-and-whisker plots depict expression of HLA class II, ICAM-1, LFA-3, OX40L and 4-1BBL on monocyte surface in medium (squares), or in the presence of IL-10, for 18 h (triangles) ($n = 5$). (b) LFA-3 mRNA expression was determined by quantitative RT–PCR in isolated monocytes cultured for 18 h in medium (white bar) and in the presence of IL-10 (black bar). Results were normalised with regard to mRNA expression in medium. (c) Box-and-whisker plots depict surface expression of CD2, LFA-1, OX-40 and 4-1BB in $CD4^+CD28^{null}$ T-lymphocytes in medium (squares) or in the presence of IL-10 (100 ng/mL) (circles) and stimulated with anti-CD3 (1 μg/mL) for 18 h. (d) PBMCs were cultured in the presence of blocking antibodies against HLA class II (2 μg/mL) and LFA-3 (2 μg/mL) before being stimulated with anti-CD3 (1 μg/mL) ($n = 5$). Percentages of Ki-67^+ and TNF-positive cells in the $CD4^+CD28^{null}$ and $CD4^+CD28^+$ T-lymphocyte subsets in these representative experiments are shown in the dot plots ($n = 5$). (e) Box-and-whisker plots represent the percentage of Ki-67^+ and TNF-positive cells in the $CD4^+CD28^{null}$ T-lymphocyte subset, in the presence of various blocking antibodies against the costimulatory molecules. In all cases, individual values are shown in scatter plots. Statistics: paired-samples *t* tests (a–c); Friedman and Wilcoxon test (e) (only comparisons with respect to the 'no-blocking antibody' condition are shown). $^*p < 0.05$; $^{**}p < 0.01$. n.s. = not significant.

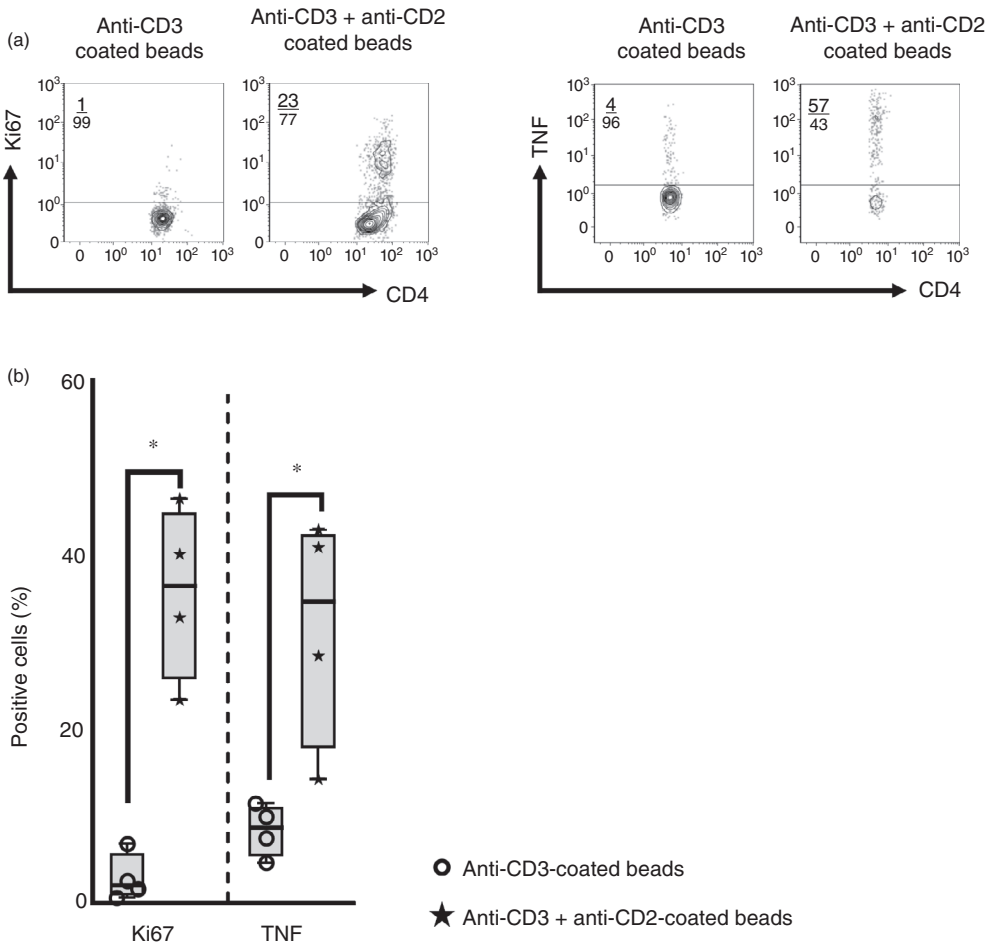

FIGURE 5 Effect of IL-10 in proliferation and cytokine production in CD4^{+}CD28^{null} T-lymphocytes costimulated through the CD2 receptor. Isolated CD4^{+} T-lymphocytes were cultured in medium, or in the presence of IL-10 (100 ng/mL), for 18 h before being stimulated with anti-CD3 + anti-CD2-coated beads ($n = 4$). Anti-CD3-coated beads without anti-CD2 were used as negative control. (a) Percentages of Ki-67^{+} and TNF- positive cells in the CD4^{+}CD28^{null} T-lymphocyte in this representative experiment are indicated in the dot plot. (b) Box-and-whisker plots show the percentages of Ki-67^{+} and TNF-positive CD4^{+}CD28^{null} T-lymphocytes with anti-CD3-coated beads (circles), anti-CD3 + anti-CD2-coated beads (star) ($n = 4$). Individual values are shown as scatter plots. Wilcoxon tests were used to compare the conditions. *$p < 0.05$.

CD4^{+}CD28^{null} T-lymphocytes under our experimental conditions. IL-10 induces T-lymphocyte inhibition by blocking the CD28 costimulatory signal [30], which cannot act in CD28^{null} T-lymphocytes, and by suppressing CD2 signalling via SHP-1. The latter only occurs in T-lymphocytes stimulated via CD2 alone, and not when anti-CD3 is also present in the cultures [31]. In other words, we did not find a direct effect of IL-10 on stimulated CD4^{+}CD28^{null} T-lymphocytes, but we did note an indirect effect on monocyte surface molecule expression. IL-10 mainly regulates T-lymphocyte activity by inhibiting the antigen presentation of monocytes/macrophages, reducing the expression of HLA class II, costimulatory and adhesion molecules, and limiting the production of cytokines [32]. We found that HLA class II, ICAM-1 and LFA-3 reduced expression in IL-10-treated monocytes, but that the ICAM-1/LFA-1 interaction was not associated with any of the effector functions of CD4^{+}CD28^{null} T-lymphocytes. By contrast, diminished expression of HLA-DR in IL-10-treated monocytes and blocking of HLA class II molecules both induced a reduction in the responses of CD4^{+}CD28^{null} T-lymphocytes. Similar effects have been described in CD28^{+} T-lymphocytes, whereby inhibition is maintained even in the presence of IL-2, and in vitro T-lymphocyte anergy is induced [33]. Moreover, LFA-3 modulation by IL-10 on monocytes also seems to have a major role in CD4^{+}CD28^{null} T-lymphocyte inhibition. IL-10 reduced protein expression of LFA-3 in monocytes as early as 24 h after treatment, an observation that was also supported by mRNA expression. The LFA-3/CD2 pathway plays a central role in activating naïve and memory T helper cells, initiating

strong antigen-independent cell adhesion, expansion of naïve T helper cells, and induction of large amounts of IFN-γ in memory cells [34,35]. In fact, some authors have proposed that CD2 compensates for the loss of CD28 because of the relatively mild phenotype associated with CD28 deficiency [36,37]. However, the absence of both receptors produces important deficits in activation and proliferation, suggesting that at least one of them must be present in T-lymphocytes to achieve an adequate response. CD2 can act as an adhesion molecule and a signalling molecule, inducing proliferation of T-lymphocytes as well as cytokine secretion, both in a ZAP70-dependent manner [35,38]. In a similar way, CD2 may be the main costimulatory molecule in $CD4^+CD28^{null}$ T-lymphocytes. The obligatory costimulation by $CD4^+CD28^{null}$ T-lymphocytes is not fully understood, but a lack of the costimulatory receptor CD28 indicates that the expression of alternative costimulatory receptors may be involved in modulating functional responses. Thus, OX40 (CD134) and 4-1BB (CD137), which are members of the tumour necrosis factor receptor (TNFR) family, are known to be important costimulatory receptors in $CD4^+CD28^{null}$ T-lymphocytes in patients with acute coronary syndrome. Moreover, several reports have demonstrated the capacity of 4-1BB engagement to costimulated $CD8^+CD28^{null}$ T-lymphocytes [39–41]. We did not find any involvement of OX40 or 4-1BB in the functional activity of $CD4^+CD28^{null}$ T-lymphocytes of CHF patients, but were able to show that LFA-3 was involved. The LFA-3/CD2 axis has been defined as the primary costimulatory pathway for $CD8^+$ T-lymphocytes that lack CD28 [42]. Similar to the finding in $CD28^+$ T-lymphocytes, engagement of the CD2 molecule by its ligand LFA-3 clearly costimulated proliferation, cytokine production, and the effector function in these cells, but this is the first study to demonstrate its relevance in $CD4^+CD28^{null}$ T-lymphocyte functionality. We demonstrated that CD2 costimulation is sufficient to induce proliferation, and that cytokine production in anti-CD3 stimulated $CD4^+CD28^{null}$ T-lymphocytes. CD2/LFA-3 signals proved to be effective only in the presence of TCR signals, which is consistent with the well-established costimulation of $CD28^+$ T-lymphocytes via CD2.

In summary, IL-10 has an inhibitory effect on $CD4^+CD28^{null}$ T-lymphocytes that can be responsible for the circulating levels and functionality of these cells. Their inhibition is not direct, but instead occurs by the costimulation and antigen presentation arising from reduced HLA class II and LFA-3 expression in monocytes. Moreover, the CD2/LFA-3 interaction proves to be a major activating mechanism in $CD4^+CD28^{null}$ T-lymphocytes.

AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows. RA-A and MAM-G designed the study; AG-T, EB-G, B-R and RL-M prepared the protocols, collected and processed all the samples, performed or oversaw the experimental protocols and analysed data; AG-T and RA-A wrote the manuscript; BD-M and JLL selected, recruited and followed up the volunteers; MAM-G and RA-A reviewed the manuscript.

FUNDING INFORMATION

This work was supported by grant PI17/00714 from Plan Estatal de I + D + i 2013–2016, co-funded by 'Instituto de Salud Carlos III' and by 'Fondo Europeo de Desarrollo Regional (FEDER)'. EB-G is sponsored by the Principado de Asturias (Programa de Ayudas 'Severo Ochoa'; BP20-030).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Alejandra García-Torre https://orcid.org/0000-0002-8277-8718

REFERENCES

1. Appay V, Zaunders JJ, Papagno L, Sutton J, Jaramillo A, Waters A, et al. Characterization of CD4(+) CTLs ex vivo. J Immunol. 2002;168:5954–8.
2. van Leeuwen EM, Remmerswaal EB, Vossen MT, Rowshani AT, Wertheim-van Dillen PM, van Lier RA, et al. Emergence of a CD4+CD28- granzyme B+, cytomegalovirus-specific T cell subset after recovery of primary cytomegalovirus infection. J Immunol. 2004;173:1834–41.
3. Moro-García MA, Alonso-Arias R, López-Larrea C. When aging reaches CD4+ T-cells: phenotypic and functional changes. Front Immunol. 2013;4:1–12.
4. Bryl E, Vallejo AN, Weyand CM, Goronzy JJ. Down-regulation of CD28 expression by TNF-alpha. J Immunol. 2001;167:3231–8.
5. Moro-Garcia MA, Alonso-Arias R, Lopez-Vazquez A, Suárez-García FM, Solano-Jaurrieta JJ, Baltar J, et al. Relationship between functional ability in older people, immune system status, and intensity of response to CMV. Age. 2012;34:479–95.
6. Alonso Arias R, Moro-García MA, Echeverría A, Solano-Jaurrieta JJ, Suárez-García FM, López-Larrea C. Intensity of the humoral response to cytomegalovirus is associated with the phenotypic and functional status of the immune system. J Virol. 2013;87:4486–95. https://doi.org/10.1128/JVI.02425-12

7. Dumitriu IE. The life (and death) of CD4+ CD28(null) T cells in inflammatory diseases. Immunology. 2015;146:185–93.
8. Thewissen M, Somers V, Hellings N, Fraussen J, Damoiseaux J, Stinissen P. CD4 + CD28 null T cells in autoimmune disease: pathogenic features and decreased susceptibility to Immunoregulation. J Immunol. 2007;179:6514–23.
9. Koller L, Richter B, Goliasch G, Blum S, Korpak M, Zorn G, et al. CD4+ CD28(null) cells are an independent predictor of mortality in patients with heart failure. Atherosclerosis. 2013; 230:414–6.
10. Sulzgruber P, Kolle L, Winter MP, Richter B, Blum S, Korpak M, et al. The impact of CD4 + CD28 null T-lymphocytes on atrial fibrillation and mortality in patients with chronic heart failure. Thromb Haemost. 2017;117:349–56. https://doi.org/10.1160/TH16-07-0531
11. Gabryšová L, Howes A, Saraiva M, O'Garra A. The regulation of IL-10 expression. Curr Top Microbiol Immunol. 2014;380: 157–90. https://doi.org/10.1007/978-3-662-43492-5_8
12. Ouyang W, O'Garra A. IL-10 family cytokines IL-10 and IL-22: from basic science to clinical translation. Immunity. 2019; 50(4):871–91. https://doi.org/10.1016/j.immuni.2019.03.020
13. Saraiva M, Vieira POA. Biology and therapeutic potential of interleukin-10. J Exp Med. 2020;217(1):e20190418. https://doi.org/10.1084/jem.20190418
14. Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, et al. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. Immunity. 2011;34:566–78. https://doi.org/10.1016/j.immuni.2011.03.018
15. Huber S, Gagliani N, Esplugues E, O'Connor W Jr, Huber FJ, Chaudhry A, et al. Th17 cells express Interleukin-10 receptor and are controlled by Foxp3- and Foxp3+ regulatory CD4+ T cells in an Interleukin-10-dependent manner. Immunity. 2011; 34:554–65. https://doi.org/10.1016/j.immuni.2011.01.020
16. Coomes SM, Kannan Y, Pelly VS, Entwistle LJ, Guidi R, Perez-Lloret J, et al. CD4 + Th2 cells are directly regulated by IL-10 during allergic airway inflammation. Mucosal Immunol. 2017;10:150–61. https://doi.org/10.1038/mi.2016.47
17. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med. 1990;323:236–41.
18. Hedayat M, Mahmoudi MJ, Rose NR, Rezaei N. Proinflammatory cytokines in heart failure: double-edged swords. Heart Fail Rev. 2010;15:543–62.
19. Vidal-Castiñeira JR, López-Vázquez A, Díaz-Peña R, Alonso-Arias R, Martínez-Borra J, Perez R, et al. Effect of killer immunoglobulin-like receptors in the response to combined treatment in patients with chronic hepatitis C virus infection. J Virol. 2010;84(1):475–81. https://doi.org/10.1128/JVI.01285-09
20. Moro-Garcia MA, Echeverria A, Galan-Artimez MC, Suárez-García FM, Solano-Jaurrieta JJ, Avanzas-Fernández P, et al. Immunosenescence and inflammation characterize chronic heart failure patients with more advanced disease. Int J Cardiol. 2014;174:590–9.
21. García-Torre A, Bueno-García E, López-Martínez R, Rioseras B, Díaz-Molina B, Lambert JL, et al. CMV infection is directly related to the inflammatory status in chronic heart failure patients. Front Immunol. 2021;12:1–11.
22. Cassatella MA, Meda L, Gasperini S, Calzetti F, Bonora S. Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. J Exp Med. 1994;179:1695–9. https://doi.org/10.1084/jem.179.5.1695
23. Kasama T, Strieter RM, Lukacs NW, Lincoln PM, Burdick MD, Kunkel SL. Interleukin-10 expression and chemokine regulation during the evolution of murine type II collagen-induced arthritis. J Clin Invest. 1995;95:2868–76. https://doi.org/10.1172/JCI117993
24. Kaur K, Dhingra S, Slezak J, Sharma AK, Bajaj A, Singal PK. Biology of TNFα and IL-10, and their imbalance in heart failure. Heart Fail Rev. 2009;14:113–23. https://doi.org/10.1007/s10741-008-9104-z
25. Moro-García MA, López-Iglesias F, Marcos-Fernández R, Bueno-García E, Díaz-Molina B, Lambert JL, et al. More intensive CMV-infection in chronic heart failure patients contributes to higher T-lymphocyte differentiation degree. Clin Immunol. 2018;192:20–9. https://doi.org/10.1016/j.clim.2018.03.015
26. Bryl E, Vallejo AN, Matteson EL, Witkowski JM, Weyand CM, Goronzy JJ. Modulation of CD28 expression with anti-tumor necrosis factor alpha therapy in rheumatoid arthritis. Arthritis Rheum. 2005;52:2996–3003.
27. Moro-Garcia MA, Alonso-Arias R, Lopez-Larrea C. When aging reaches CD4+ T-cells: phenotypic and functional changes. Front Immunol. 2013;4:107.
28. Yung R, Powers D, Johnson K, Amento E, Carr D, Laing T, et al. Mechanisms of drug-induced lupus. II. T cells overexpressing lymphocyte function-associated antigen 1 become autoreactive and cause a lupuslike disease in syngeneic mice. J Clin Invest. 1996;97:2866–71.
29. Rioseras B, Moro-García MA, García-Torre A, Bueno-García E, López-Martínez R, Iglesias-Escudero M, et al. Acquisition of new migratory properties by highly differentiated CD4+CD28null T lymphocytes in rheumatoid arthritis disease. J Pers Med. 2021;11:1–20.
30. Akdis CA, Joss A, Akdis M, Faith A, Blaser K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. FASEB J. 2000;14:1666–8. https://doi.org/10.1096/fj.99-0874fje
31. Taylor A, Verhagen J, Akkoç T, Wenig R, Flory E, Blaser K, et al. IL-10 suppresses CD2-mediated T cell activation via SHP-1. Mol Immunol. 2009;46:622–9. https://doi.org/10.1016/j.molimm.2008.07.031
32. Salek-Ardakani S, Arrand JR, Mackett M. Epstein-Barr virus encoded interleukin-10 inhibits HLA-class I, ICAM-1, and B7 expression on human monocytes: implications for immune evasion by EBV. Virology. 2002;304:342–51. https://doi.org/10.1006/viro.2002.1716
33. Kriegel MA, Adam-Klages S, Gabler C, Blank N, Schiller M, Scheidig C, et al. Anti-HLA-DR-triggered monocytes mediate in vitro T cell anergy. Int Immunol. 2008;20:601–13.
34. Wingren AG, Parra E, Varga M, Kalland T, Sjogren HO, Hedlund G, et al. T cell activation pathways: B7, LFA-3, and ICAM-l shape unique T cell profiles. Crit Rev Immunol. 2017;

37:463–81. https://doi.org/10.1615/CritRevImmunol.v37.i2-6.130
35. Binder C, Cvetkovski F, Sellberg F, Berg S, Paternina Visbal H, Sachs DH, et al. CD2 Immunobiology. Front Immunol. 2020; 11:1–14.
36. Green JM, Karpitskiy V, Kimzey SL, Shaw AS. Coordinate regulation of T cell activation by CD2 and CD28. J Immunol. 2000;164:3591–5. https://doi.org/10.4049/jimmunol.164.7.3591
37. Kalland ME, Oberprieler NG, Vang T, Taskén K, Torgersen KM. T cell-signaling network analysis reveals distinct differences between CD28 and CD2 Costimulation responses in various subsets and in the MAPK pathway between resting and activated regulatory T cells. J Immunol. 2011;187:5233–45. https://doi.org/10.4049/jimmunol.1101804
38. Meinl E, Lengenfelder D, Blank N, Pirzer R, Barata L, Hivroz C. Differential requirement of ZAP-70 for CD2-mediated activation pathways of mature human T cells. J Immunol. 2000;165:3578–83. https://doi.org/10.4049/jimmunol.165.7.3578
39. Bukczynski J, Wen T, Watts TH. Costimulation of human CD28- T cells by 4-1BB ligand. Eur J Immunol. 2003;33:446–54. https://doi.org/10.1002/immu.200310020
40. Waller ECP, McKinney N, Hicks R, Carmichael AJ, Sissons JGP, Wills MR. Differential costimulation through CD137 (4-1BB) restores proliferation of human virus-specific "effector memory" (CD28- CD45RA HI) CD8+ T cells. Blood. 2007;110:4360–6.
41. Kober J, Leitner J, Klauser C, Woitek R, Majdic O, Stöckl J, et al. The capacity of the TNF family members 4-1BBL, OX40L, CD70, GITRL, CD30L and LIGHT to costimulate human T cells. Eur J Immunol. 2008;38:2678–88. https://doi.org/10.1002/eji.200838250
42. Leitner J, Herndler-Brandstetter D, Zlabinger GJ, Grubeck-Loebenstein B, Steinberger P. CD58/CD2 is the primary costimulatory pathway in human CD28—CD8 + T cells. J Immunol. 2015;195:477–87. https://doi.org/10.4049/jimmunol.1401917

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: García-Torre A, Bueno-García E, Moro-García MA, López-Martínez R, Rioseras B, Díaz-Molina B, et al. IL-10 indirectly modulates functional activity of $CD4^{+}CD28^{null}$ T-lymphocytes through LFA-3 and HLA class II inhibition. Immunology. 2024. https://doi.org/10.1111/imm.13824