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RESEARCH ARTICLE

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PTPN22 1858C>T polymorphism is associated with increased CD154 expression and higher CD4+ T cells percentage in rheumatoid arthritis patients

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Abstract

Background: CD40 is a costimulatory molecule for B cells, and CD154 is a marker of CD4+ T cells activation. CD40-CD154 interaction promotes pro-inflammatory cytokines secretion and autoantibodies production. *PTPN22* gene encodes LYP protein, an inhibitor of T- and B-cell activation. *PTPN22* 1858C>T polymorphism confers rheumatoid arthritis (RA) susceptibility. Hence, we evaluate the relationship between 1858C>T polymorphism with CD40 and CD154 expression and IFN-γ secretion in RA patients.

Methods: *PTPN22* 1858C>T polymorphism was genotyped in 315 RA patients and 315 control subjects (CS) using PCR-RFLP method. Later, we selected only ten anti-CCP-positive RA patients, naïve to disease-modifying antirheumatic drugs and ten CS, all with known 1858C>T *PTPN22* genotype. The CD40 and CD154 membrane expressions were determined by flow cytometry in peripheral B and T cells, correspondingly.

Results: The B cells percentage and mCD40 expression were similar between RA and CS ($P > 0.05$) and we did not find an association between these variables and the 1858C>T polymorphism. The CD4+ T cells percentage was higher in RA patients than CS ($P = 0.003$), and in the RA group, the CD4+ T cells percentage and mCD154 expression were higher in the 1858 T allele carriers ($P = 0.008$ and $P = 0.032$, respectively). The IFN-γ levels were lower in RA patients carrying the *PTPN22* risk allele ($P = 0.032$).

Conclusion: The *PTPN22* 1858 T risk allele is associated with increased CD4+ T cells percentage and high mCD154 expression in RA patients, which could favor the pro-inflammatory cytokine release and the establishment of the inflammatory response at the seropositive RA.

KEYWORDS
anti-CCP positive, CD154, CD40, IFN-γ, PTPN22, rheumatoid arthritis

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1 | INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease, characterized by immune cell-mediated destruction of the joints. Hyper-reactive T and B lymphocytes are key cells in the destructive process by producing inflammatory cytokines and autoantibodies.[1] The protein tyrosine phosphatase non-receptor type 22 gene (*PTPN22*) encodes the lymphoid protein tyrosine phosphatase LYP, which is expressed exclusively in hematopoietic cells, including T and B cells.[2] LYP is a potent negative regulator of T and B lymphocyte activation by modulating T-cell receptor (TCR) and B-cell receptor (BCR) signaling.[3-6]

A functional single nucleotide polymorphism (SNP) 1858C>T (rs2476601) in the *PTPN22* gene leads to the 620Arg>Trp substitution within the first pro-rich region in the C-terminus of LYP and has been consistently associated with RA susceptibility and anti-CCP antibodies seropositivity.[7-9] In the western Mexican population, this polymorphism has been found in low frequency (1858 T allele, 6%), however, it has been consistently associated with RA risk.[8,10]

The mechanism of increased risk by the T allele remains unclear and controversial results have been exposed. Some authors have shown that this polymorphic allele (1858 T or 620Trp) is a gain-of-function variant, displaying a more potent inhibition on TCR and BCR signaling.[11,12] On the other hand, it has also been shown that the same allele is a loss-of-function variant as it favors a hyperresponsive phenotype on several immune cells including T and B lymphocytes, and therefore, it is associated with the development of autoimmunity.[7,13-16]

A study reported that the 1858 T risk allele interferes with the removal of developing autoreactive B cells and it is associated with an increase in CD40 expression on naïve B-cell surface.[17] CD40 is a co-stimulatory protein constitutively expressed on B cells, which promotes B-cell proliferation and differentiation, germinal center formation and antibody production by its interaction with its ligand, CD154.[18]

Furthermore, CD154 is expressed on the surface of activated T cells and is upregulated faster and to a higher degree in peripheral blood and synovial T cells from RA patients as compared to healthy controls, with the consequent increased production of pro-inflammatory cytokines such as IFN-γ.[19,20] In addition, overexpression of CD154 on T cells correlates with higher RA activity.[21] To date, the functional role of the *PTPN22* 1858C>T polymorphism in autoimmune diseases is not entirely clear and its precise impact on signaling pathways is highly context dependent.[6] Since the CD40/CD154 interaction promotes B- and T-cell activation and cytokine secretion, the aim of this study was to evaluate the relationship between the 1858 T risk allele with CD40 and CD154 expression and IFN-γ secretion in anti-CCP-positive RA patients.

2 | MATERIALS AND METHODS

2.1 | Study subjects and *PTPN22* genotyping classification

In a previous study,[10] we determined the genotypes of *PTPN22* 1858C>T SNP in 315 RA patients and 315 control subjects from western Mexico using PCR-RFLP method. Participants were classified into two groups: carriers, for those who had at least one copy of the RA risk-conferring T allele (1858CT, 1858 TT), and not carriers, for those who were homozygous for the non-risk-conferring allele (1858CC). Taking these data into account, and considering the low frequency of this polymorphism, for this study, we selected a very specific cohort of patients, which consisted of the following: ten RA patients with an onset of maximum two years of clinical symptoms, positive for anti-CCP antibodies, and naïve to disease-modifying antirheumatic drugs (DMARDs). Ten CS with no family history of autoimmunity and no clinical signs of autoimmune or infectious disease, and negative for anti-CCP antibodies also were included.

This study was realized according to the Declaration of Helsinki. The ethics and biosecurity committee of the "University Center for Health Science, University of Guadalajara," approved this study and all subjects provided informed consent before their inclusion. A blood sample was obtained from every subject, from which peripheral blood mononuclear cells (PBMCs) and serum were obtained.

2.2 | Flow Cytometry for CD40 and CD154 molecules

Murine anti-human monoclonal antibodies (mAbs) anti-CD19-FITC, anti-CD4-FITC, anti-CD40-PE, anti-CD154-PE, and their respective isotypic control mAbs (all from Biolegend, Inc) were used to determine CD40 expression on $CD19^+$ B cells and CD154 expression on $CD4^+$ T cells, correspondingly. PBMCs from RA patients and CS were incubated with the manufacturer's recommended concentrations of corresponding antibodies for 30 minutes at 4°C. After incubation, erythrocytes were lysed using 1X BD FACS Lysis Buffer (BD Biosciences) followed by centrifugation and vortex steps; the resulting cells were resuspended in a phosphate-buffered saline (PBS)/0.5% formaldehyde solution. Two-color flow cytometry assay was performed using a Beckman Coulter flow cytometer. First, lymphocytes were gated according to their forward scatter (FS) and side scatters (SS) characteristics. Then, further gates were placed around those CD4+ T cells or CD19+ B cells. Subsequently, for each sample, the percentages of positive cells for CD19/CD40 and CD4/CD154, as well as the mean fluorescence intensity (MFI) of CD40 and CD154, were recorded. Results were analyzed by means of Flow Jo v.10.0.6 software.

2.3 | IFN-γ quantification

Peripheral blood [5 mL] was collected and centrifuged at 300 g for 15 minutes to obtain the serum, which was stored at −70°C until processing by ELISA assays. Serum interferon gamma (IFN-γ) levels were analyzed using a commercially available ELISA kit (R&D Systems) conforming to the manufacturer's instructions. Detection limit for the assay was 0.8 pg/mL.

2.4 | Statistics

Kolmogorov-Smirnov test was used to evaluate if the data sets were normally distributed. Resulting CD40 and CD154 percentage expression and MFI, as well as IFN-γ serum levels, were expressed

TABLE 1 Demographic and clinical characteristics of study subjects

	RA (n = 10)	CS (n = 10)	*P* value
Demographics			
Age (y)[a]	49 (33-65)	46 (25-70)	0.59
Sex (Female/Male)	9/1	8/2	
Clinical			
Disease duration (years)[a]	0.8 (0.1-1.5)	-	NA
Patient´s global assessment of disease status (0-10 VAS)[b]	4.5 ± 3.3	-	NA
DAS28[c]	3.3 ± 0.8	-	NA
Autoantibodies			
RF (IU/mL)[b]	118.3 ± 40.10	5.2 ± 1.9	<0.001
Positive (>30 IU/mL)[c]	70 (7)	0 (0)	
Anti-CCP (U/mL)[b]	35.2 ± 17.6	2.7 ± 1.6	<0.0001
Positive (>5 U/mL)[c]	100 (10)	0 (0)	

RA, rheumatoid arthritis; CS, control subjects; VAS, visual analog scale; DAS28, disease activity score using 28 joint counts; RF, rheumatoid factor; Anti-CCP, anti-cyclic citrullinated peptide antibodies; NA, not applicable.
[a]Data presented as median (min-max).
[b]Data provided as mean ±SD.
[c]Data provided as percentage and n.

as median and interquartile range (IQR). The data were analyzed by Mann-Whitney *U* test; a *P*-value <0.05 was considered statistically significant. All statistical analysis was performed by means of SPSS v.20 software (SPSS, Chicago, IL, USA) and GraphPad Prism 6.0c.

3 | RESULTS

3.1 | CD40 is not differentially expressed on B cells carrying the *PTPN22* risk allele

Our study enrolled ten anti-CCP-positive RA patients and ten CS, all with known *PTPN22* 1858C>T genotype. The demographic and clinical characteristics of these groups are shown in Table 1. The median age for RA patients and CS was similar (49 and 46 years, respectively, $P > 0.05$). RA patients had a median disease duration of 8 months, and their disease activity was considered as moderate conforming to DAS28 score.

To analyze the impact of *PTPN22* on the CD40 expression in B cells, we quantified the $CD19^+/CD40^+$ B-cell percentage in PBMCs and then we measured membrane CD40 (mCD40) expression using flow cytometry (Figure 1A,B).

$CD19^+/CD40^+$ B-cell percentage was not significantly different among CS and RA patients [median (IQR): 7.41 (6.65-11.25) vs 7.86 (6.62-11.83), respectively; $P = 0.345$] (Figure 1C). Furthermore, when comparing $CD19^+/CD40^+$ B-cell percentage between RA patients carrying or not the *PTPN22* risk allele, no difference was obtained [10.70 (7.10-14.30) vs 6.77 (5.39-11.17), respectively; $P = 0.222$] (Figure 2A); the same was observed for comparisons of CS (Figure 2C) and CS +RA (Figure 2E) classified as carriers or non-carriers of the risk allele.

There was no statistically significant difference in CD40 expression among RA patients and CS (data not shown). In RA, we only detected a trend toward a higher expression in non-carriers of T allele versus carriers [57.90 (50-40-102.70) vs 46.50 (32.80-58.05), respectively; $P = 0.095$] but no significance was achieved (Figure 2B). Similar findings were observed when we made the same comparison in the CS (Figure 2D) or RA +CS group (Figure 2E, $P > 0.05$). Our results show that the *PTPN22* 1858C>T polymorphism is not associated for variations in CD40 expression on B-cell surface.

3.2 | Carriers of the *PTPN22* risk allele have increased counts of activated T cells and increased expression of mCD154

We calculated the percentages of activated T helper cells in PBMCs of the study subjects, measured as the total of cells positive for the CD4 and CD154 markers simultaneously ($\%CD4^+/CD154^+$) (Figure 1D,E). Then, we established comparisons between RA patients and CS, and found significant differences between them, since RA patients displayed a marked increase in the frequency of these cells [CS: 1.63 (0.67-1.79); RA: 2.45 (1.88-5.18); $P = 0.003$] (Figure 1F).

The same analysis was performed in the RA group classified according to their 1858C>T *PTPN22* genotype. We detected a higher frequency of activated $CD4^+$ T cells in patients carrying the risk allele over patients not carrying the allele [4.81 (2.91-7.95) vs 1.93 (1.70-2.22); $P = 0.008$] (Figure 3A). Similar results were obtained when comparing CS (Figure 3C) and CS +RA (Figure 3E) classified as carriers or non-carriers.

Consequently, we analyzed the expression of mCD154 on $CD4^+$ T cells from CS and RA but no significant differences were

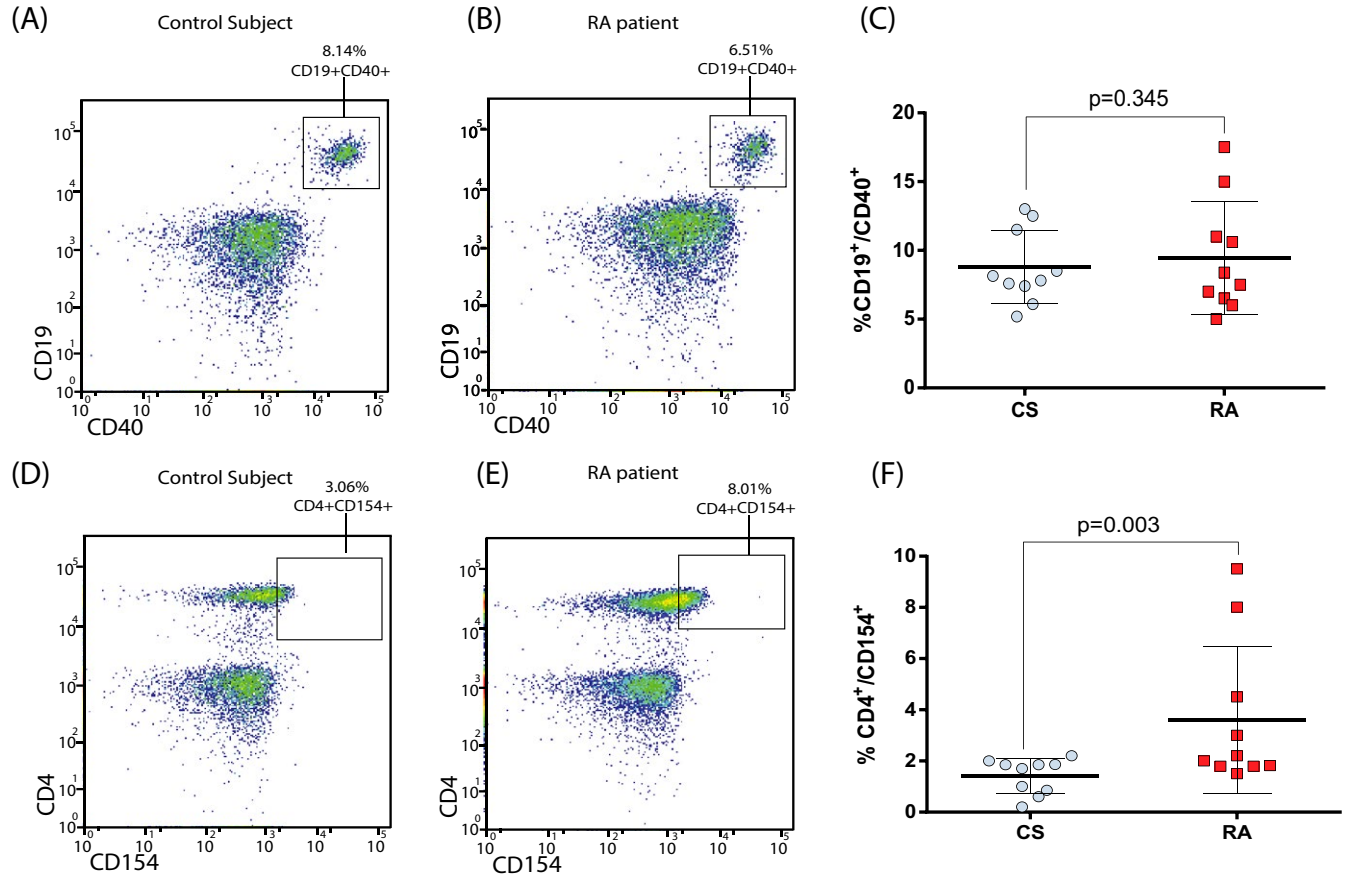

FIGURE 1 Circulating T and B lymphocytes in CS and RA patients. All analyses were conducted in ten RA patients and ten CS. Two-color flow cytometry analysis shows a representative graph of the percentage of B cells ($CD19^+/CD40^+$) in the control group (A) and anti-CCP^+ patients with RA (B); (C) Distribution (percentage) of B cells in controls and anti-CCP^+ patients with RA. In D, E we show a representative example of the activated T helper cells ($CD4^+/CD154^+$) percentage in the CS group and anti-CCP^+ patients with RA, respectively; (F) Distribution (percentage) of T cells in controls and anti-CCP^+ patients with RA. Comparison among groups was performed using Mann-Whitney *U* test

detected among groups [1.20 (0.89-1.45) vs 1.49 (1.22-1.78), respectively; $P = 0.1883$] (data not shown). However, within the RA group, mCD154 expression was higher in patients bearing the risk allele than in non-carriers [1.44 (1.17-1.72) vs 0.91 (0.07-1.20), respectively; $P = 0.032$] (Figure 3B). The same results were obtained when comparing CS (Figure 3D) and CS +RA patients, classified conforming to their genotypes.

Our results point out that the 1858 T *PTPN22* allele is associated with a higher number of activated CD4+ T cells and an increased expression of mCD154.

3.3 | *PTPN22* 1858 T risk allele is associated with decreased IFN-γ serum levels

IFN-γ serum levels were measured in all subjects included. A borderline difference was detected when comparing CS levels versus RA levels regardless of their genotypes, being increased in RA patients [CS: 0.64 (0.07-3.17) pg/mL; RA: 3.27 (2.03-10.25) pg/mL; $P = 0.05$] (data not shown).

When comparing IFN-γ levels in RA patients classified in accordance with their *PTPN22* genotype, significantly higher levels were detected in the non-carrier group [carriers: 2.38 (0.57-3.27) pg/mL vs non-carriers 8.54 (3.29-17.73) pg/mL; $P = 0.02$, Figure 4A]. Intriguingly, the opposite was observed when the comparison was performed in CS (Figure 4B); there was a higher production of IFN-γ in CS carrying the risk allele, over those not carrying it [4.10 (2.86-5.12) pg/mL vs 0.36 (0.07-0.86) pg/mL, respectively; $P = 0.03$]. When combining CS and RA patients (Figure 4C) and classifying according to their genotypes, the difference was lost [carriers: 2.83 (1.34-4.00) pg/mL; non-carriers: 1.34 (0.14-7.39) pg/mL; $P = 0.46$].

4 | DISCUSSION

The missense variant (1858C>T) of the protein tyrosine phosphatase non-receptor 22 (*PTPN22*) gene has one the strongest effects among RA-associated common variants, and it has been associated with

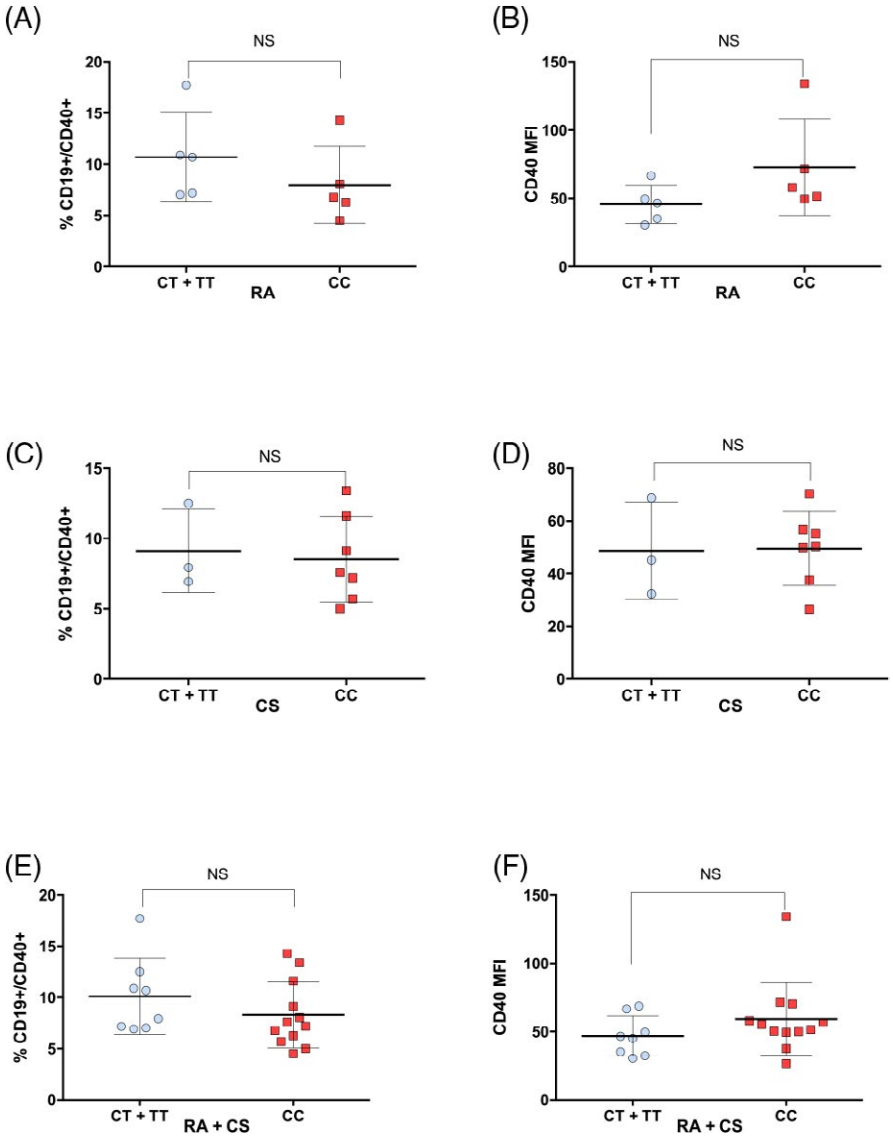

FIGURE 2 *PTPN22* does not influence CD40 expression on B cell. Peripheral blood mononuclear cells (PBMCs) from RA patients and healthy donors were incubated with anti-CD19 antibody and anti-CD40 antibody, with FITC and PE-conjugated fluorochromes. A, Percentage of $CD19^+/CD40^+$ B cell in anti-CCP^+ RA patients grouped as carriers (n = 5) and non-carriers (n = 5) of 1858 T risk allele of *PTPN22* gene. B, CD40 MFI in B cells of anti-CCP^+ RA patients grouped as carriers (n = 5) and non-carriers (n = 5) of 1858 T risk allele of *PTPN22* gene. C, Percentage of $CD19^+/CD40^+$ B cell in CS grouped as carriers (n = 3) and non-carriers (n = 7) of 1858 T risk allele of *PTPN22* gene. D, CD40 MFI in B cells of CS grouped as carriers (n = 3) and non-carriers (n = 7) of 1858 T risk allele of *PTPN22* gene. E, Percentage of $CD19^+/CD40^+$ B cell in anti-CCP^+ RA patients and CS combined and grouped as carriers (n = 8) and non-carriers (n = 12) of 1858 T risk allele of *PTPN22* gene. F, CD40 MFI in B cells of anti-CCP^+ RA patients and CS combined and grouped as carriers (n = 8) and non-carriers (n = 12) of 1858 T risk allele of *PTPN22* gene

over 20 different autoimmune diseases.[22] Surprisingly, the mechanisms underlying the association between *PTPN22* and autoimmune diseases are still largely unknown.

The first study evaluating the association between RA and 1858C>T SNP reported an association only with rheumatoid factor positive RA, pointing out the possibility that *PTPN22* 1858 T allele affects autoantibody production.[7] These findings are consistent with our findings reported elsewhere.[8]

In vitro assays have shown that the 1858 T risk allele leads to interference with the physical association between LYP and c-Src kinase (CSK), resulting in increased LYP activity. Supporting these findings, both TCR signaling and BCR signaling have been found to be reduced in the lymphocytes of risk allele carriers.[11,12,23]

The interaction of the CD40 and CD154 molecules plays a significant role in T- and B-cell activation, as it increases cytokine production by T cells, as well as isotype change and antibody production by B lymphocytes.[24] Several authors report increased expression and soluble levels of these molecules in autoimmune diseases.[18]

A previous study shows that the 1858 T allele of the *PTPN22* gene is associated with increased CD40 expression on the naïve B-cell membrane of healthy subjects.[17] Therefore, in this study, we were interested in studying the effect of the 1858C>T *PTPN22* polymorphism on the CD40 and CD154 expression in B and T cells membrane, correspondingly, as well as serum levels of IFN-γ in anti-CCP-positive RA patients.

Although in other autoimmune diseases such as systemic lupus erythematosus (SLE), several studies have shown differences in certain peripheral B-cell subsets compared to healthy subjects,[25] we did not find significant differences in the B cells percentage and CD40 MFI between CS and RA patients. In line with our results, it was accepted in consensus that there is a lack of differences on total peripheral B cells among RA patients and CS[26]; in addition, a study has shown that the number of various subpopulations of peripheral B cells is not different among RA patients classified according to their

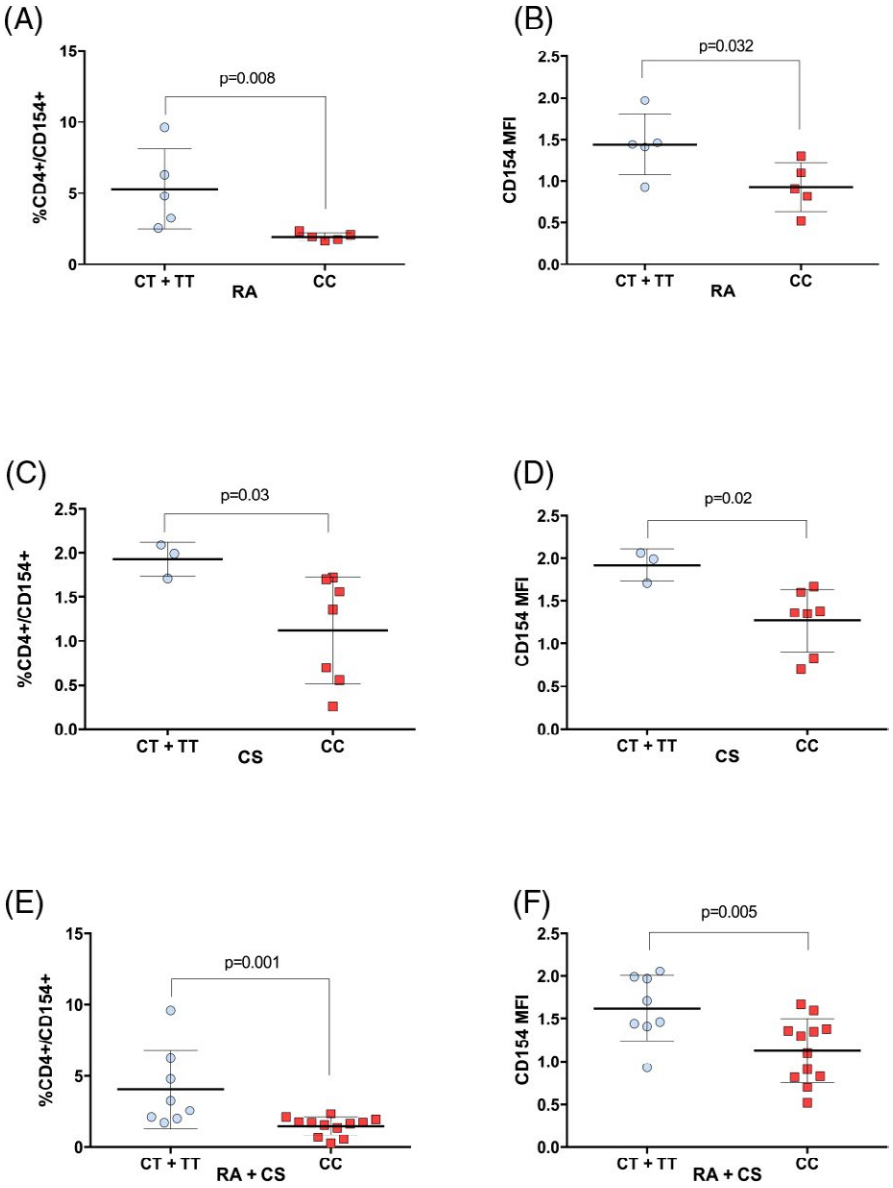

FIGURE 3 *PTPN22* increases amounts of activated T cells and CD154 expression levels on T helper cell. Peripheral blood mononuclear cells (PBMCs) from RA patients and healthy donors were incubated with anti-CD4 antibody and anti-CD154 antibody, with FITC and PE-conjugated fluorochromes. A, Percentage of $CD4^+/CD154^+$ T cell in anti-CCP^+ RA patients grouped as carriers (n = 5) and non-carriers (n = 5) of 1858 T risk allele of *PTPN22* gene. B, CD154 MFI in T cells of anti-CCP^+ RA patients grouped as carriers (n = 5) and non-carriers (n = 5) of 1858 T risk allele of *PTPN22* gene. C, Percentage of $CD4^+/CD154^+$ T cell in CS grouped as carriers (n = 3) and non-carriers (n = 7) of 1858 T risk allele of *PTPN22* gene. D, CD154 MFI in T cells of CS grouped as carriers (n = 3) and non-carriers (n = 7) of 1858 T risk allele of *PTPN22* gene. E, Percentage of $CD4^+/CD154^+$ T cell in anti-CCP^+ RA patients and CS combined and grouped as carriers (n = 8) and non-carriers (n = 12) of 1858 T risk allele of *PTPN22* gene. F, CD154 MFI in T cells of anti-CCP^+ RA patients and CS combined and grouped as carriers (n = 8) and non-carriers (n = 12) of 1858 T risk allele of *PTPN22* gene

seropositivity to FR and anti-CCP antibodies and patients with synovitis.[27] Nevertheless, when studying cells residing on lymph nodes, a significant increase in the number of B cells in RA patients as compared to CS has been demonstrated.[28]

B cells are critical players in human immune responses including autoimmune conditions; however, it is well known that in SLE, there is a predominance of a humoral response mediated by B cells compared to RA, thus, this explains why these data are not equally robust in RA.

Although our results suggest that there are not a higher number of B lymphocytes in stages of RA, we do not discard that the assessments in the peripheral blood may present minor sensitivity and this sample could not totally be representative of the pathophysiological status in the synovial tissue. In addition, it has been reported that CD40 expression is increased on the B-cell membrane of RA patients as compared to CS,[29] and a possible explanation for the discrepancy in our results might reside on the course of the disease (<2 years) of our study group; at this stage, perhaps the difference in CD40 expression is only detectable on cells residing in lymph nodes.

There is only one study suggesting that B-cell tolerance defects in autoimmunity can result from specific genetic variants, such as the 1858 T allele of the *PTPN22* gene and precedes the onset of disease. These authors demonstrated an increase in CD40 expression on naïve B cells of healthy subjects carrying the T allele.[17] In RA patients, we did not observe significant differences in the percentages or expression of CD40 on B cells conforming their genotype, and the same was observed when only CS as well as RA patients and CS combined were analyzed. Nevertheless, it is important to point out that the analyzed cells in that study were mature naïve B cells, whereas on our study total peripheral B cells were evaluated. Since the behavior of different cell subpopulations varies according to their maturation and activation status, this could explain the differences observed. Thus, our results suggest that the *PTPN22* 1858 T allele does not affect the expression of CD40 on total peripheral B cells.

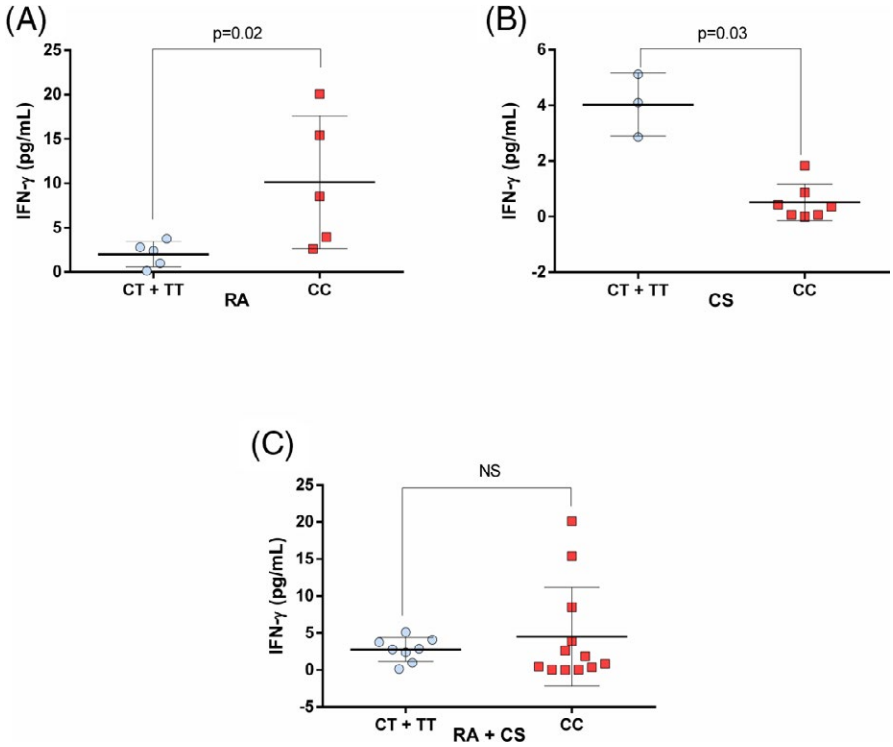

FIGURE 4 *PTPN22* 1858 T risk allele association with serum levels of IFN-γ. Serum IFN-γ levels were analyzed using commercially available ELISA kit. A, Comparison of IFN-γ serum levels between RA patients classified as carriers (n = 5) and non-carriers (n = 5) of *PTPN22* 1858 T allele. B, Comparison of IFN-γ serum levels between CS classified as carriers (n = 3) and non-carriers (n = 7) of *PTPN22* 1858 T allele. C, Comparison of IFN-γ serum levels between RA patients and CS combined and classified as carriers (n = 8) and non-carriers (n = 12) of *PTPN22* 1858 T allele

With regard to the percentage of CD154+ T cells, a relevant finding of our study was that the percentage of activated T cells was higher in RA patients than in CS. Our results are consistent with the ongoing inflammatory process in RA patients in which an increased number of CD4+/CD154+ activated T cells that produce cytokines and chemokines and favor the production of antibodies, which is expected. These results are consistent with previous reports demonstrating an increase in the number of peripheral blood $CD4^+$ T cells in RA patients.[30]

Although we detected an increase in the number of CD154+/CD4+ T cells in RA patients, single cell expression of CD154+ as evaluated by MFI was not different between RA patients and CS. This is opposite to previous reports showing an increase in CD154 expression on the membrane of CD4+ T cells of RA patients.[31] These differences may be due to the disease activity score of our patients since it has been proposed that CD154 expression is directly associated with disease activity[21] which is evidenced, as we could not detect any differences because our group displayed a mean moderate activity.

Importantly, in the anti-CCP-positive RA group we observed an increase in the ($CD4^+$/$CD154^+$) activated T cells percentage and CD154 expression in those individuals carrying the 1858 T risk allele; this was significant both in RA patients and in CS evaluated separately as well as combined. To our knowledge, this is the first report associating CD154 expression on T cells to *PTPN22* genotype. Our results suggest that the *PTPN22* 1858 T allele leads to an increase in both T helper cell activation and proliferation and that it might be acting as a promoter of an autoimmune scenario even before any clinical evidence of autoimmunity; according to that, models in T cells have been proposed which explain that the T risk allele results in impaired binding between LYP and the SH3 (Src homology 3 domain) of CSK,[32,33] which is an important suppressor of kinases that mediate T-cell activation. Another possible effect of the *PTPN22* 1858 T allele on T cells is that found by a recent study, which reported that upon TCR-activation, naïve human CD4+ T cells from homozygous for the *PTPN22* risk allele overexpress a set of genes including *CFLAR* and *4-1BB*, which are important for cytotoxic T-cell differentiation. Moreover, they found an accumulation of a subgroup of CD4+ T cells producing perforin-1 (EOMES+CD4+ T cells) in synovial fluid of RA patients in *PTPN22* risk allele carriers,[34] and these cells were proposed as a relevant T-cell subset in RA pathogenesis. Altogether, these findings and ours provide novel mechanisms of action of the *PTPN22* risk allele in RA.

Activated CD4+ T cells release high IFN-γ levels[35]; this cytokine enhances chemokine expression for leukocyte recruitment by facilitating their transfer through the endothelial layer, increases antigen presentation, and promotes Th1 differentiation,[36] all of these functions are preponderant in the pathophysiology of RA. We observed a borderline significant increase in the IFN-γ serum levels of RA patients in relation to CS, which is in agreement with previous studies.[37,38]

Respecting to 1858C>T genotype, we observed that in the RA group, the presence of the risk allele favors a significant decrease in IFN-γ but intriguingly, in CS it is associated with an increase in IFN-γ levels. In this respect, a group of researchers documented that after in vitro antigen stimulation, $CD4^+$ T cells from healthy donors carriers of 1858 T risk allele produced significantly higher amounts of IFN-γ and TNF-α than subjects carrying wild-type allele.[39] However, another study has shown in a case report that a type 1 diabetes patient with 1858 TT *PTPN22* genotype has

diminishing T-cell proliferation and reduced IFN-γ production.[40] This allows us to suggest that possibly the real effect of this polymorphism is that observed in control groups and that the discrepancy observed in RA patients could be because of other immunoregulatory cytokines, which may be elevated in response to the increase of IFN-γ caused by the T allele. It is also possible that the canonical signaling pathway behind IFN-γ induction is altered in RA. Our results suggest that when evaluating the effect of this polymorphism, not only the cell status should be considered, but the disease status as well, since several other factors and interactions may be involved that provoke different responses in patients as compared to healthy subjects.

The frequency of the *PTPN22* 1858 T allele varies considerably across the world; European populations have shown a higher frequency than other populations,[41] the T allele is virtually absent in Han Chinese and Africans, whereas in African Americans the risk allele has been detected in intermediate frequencies (2.4%).[42] In the western Mexican population, the allelic frequency of this polymorphism is low (1858 T allele, 6%), which can be reflected in the low genotypic frequencies (CC = 96%, CT = 4%, and TT = 0%) observed at the general population.[8]

One of the weaknesses of this study is that in our population, we did not find individuals homozygous for the polymorphic variant; therefore, this makes it impossible to evaluate the effect of this polymorphism in a double dose. Also, we do not evaluate additional T-cell activation markers (eg, CD69, CD45RO) to verify the activation status of CD4+/CD154+ T cells.

In conclusion, our results suggest that the role of the 1858C>T *PTPN22* polymorphism in the induction of autoimmunity could be based on the increased activation and proliferation of T helper cells, favoring the establishment of the autoimmune process in RA. This polymorphism does not seem to have relevance in the activation or differentiation of B cells since it does not act directly on these cells in patients with RA positive to anti-CCP antibodies.

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CONFLICT OF INTEREST

Authors declare that they had no conflict of interest.

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