EXTENDED REPORT

B cell resistance to Fas-mediated apoptosis contributes to their ineffective control by regulatory T cells in rheumatoid arthritis

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ABSTRACT

Objective To investigate whether regulatory T cells (Treg) can control B cell function in rheumatoid arthritis (RA) and if not to explore the basis for this defect.

Methods Suppression of B cell responses by Treg was analysed in vitro by flow cytometry and ELISA using peripheral blood mononuclear cells from 65 patients with RA and 41 sex-matched and aged-matched healthy volunteers. Blocking and agonistic antibodies were used to define the role of Fas-mediated apoptosis in B cell regulation.

Results Treg failed to restrain B cell activation, proinflammatory cytokine and antibody production in the presence of responder T cells in RA patients. This lack of suppression was not only caused by impaired Treg function but was also due to B cell resistance to regulation. In healthy donors, control by Treg was associated with increased B cell death and relied upon Fas-mediated apoptosis. In contrast, RA B cells had reduced Fas expression compared with their healthy counterparts and were resistant to Fas-mediated apoptosis.

Conclusions These studies demonstrate that Treg are unable to limit B cell responses in RA. This appears to be primarily due to B cell resistance to suppression, but Treg defects also contribute to this failure of regulation. Our data identify the Fas pathway as a novel target for Treg-mediated suppression of B cells and highlight a potential therapeutic approach to restore control of B cells by Treg in RA patients.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease with a significant morbidity and reduced life expectancy. Although substantial therapeutic progress has been achieved with the use of biological therapies, a cure remains elusive. B cells are considered to play a pivotal role in the pathology of this disease,[1 2] not least the therapeutic success of rituximab, which depletes them in vivo.[3] Although the precise contribution of B cells to RA pathogenesis remains unclear, their potential roles are multiple.[1 2 4] B cells are potent antigen presenting cells and efficiently drive autoimmune T cell responses. They secrete inflammatory cytokines and chemokines, recruiting further cells to the inflammatory site. Finally, they give rise to plasma cells, which produce a variety of autoantibodies. These pathogenic properties prompted us to determine if B cells have escaped control from regulatory T cells (Treg) in RA.

In healthy individuals, Treg can suppress numerous cell types, preventing damaging autoimmune responses.[5] We and others have shown that Treg function is impaired in RA with respect to suppression of CD4 T cells,[6–8] but there are no data indicating whether B cells are kept in check in this disease. Treg can limit autoreactive B cell responses in mice: Treg removal results in aberrant autoantibody production and worsens arthritis in susceptible models, whereas Treg administration reverses these effects.[9–11] FoxP3-deficient mice exhibit altered B cell development and a failure in B cell anergy.[12] In the absence of Treg, B cell loss of tolerance was driven by responder T cells.[12] In fact, self-perpetuating B/responder T cell interactions play a key role in systemic autoimmune disease.[13] CD4 T cell depletion prevents the development of autoreactive B cells, whereas provision of CD4 help is sufficient to activate anergic autoreactive B cells.[14] The genetic associations of the HLA-shared-epitope alleles with anti-cyclic citrullinated peptide antibodies lends further support that responder T cells provide crucial help to autoimmune B cells in RA.[15] Therefore, we studied the potential for Treg to restrain B cell responses in the presence of responder T cells. We investigated Treg control of B cells in healthy donors and RA patients and explored the underlying mechanisms involved.

MATERIALS AND METHODS

Subjects

Blood samples were obtained from healthy volunteers and patients attending the rheumatology clinic at University College London Hospital and fulfilling the revised classification criteria of the American College of Rheumatology for RA. Patients receiving no treatment or non-biological therapy only were selected. They were seropositive and had active disease with a disease activity score (DAS28) of >5.1.

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were separated on Ficoll-Paque (GE-Healthcare) gradient centrifugation and stored in liquid nitrogen. Treg were depleted from PBMCs, or B cells, responder T cells and Treg were isolated by flow cytometry sorting (Aria, BD) (see online supplementary figure S1).

Treg depletion assay

0.3×10^6 cells/well of PBMCs or Treg-depleted PBMCs were stimulated with 0.5 µg/mL soluble anti-CD3 antibody (HIT3a, eBioscience). The %

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change after Treg depletion was calculated as follows: [100×(% B cell expression in Treg-depleted PBMCs)/(% B cell expression in PBMCs)]−100

Treg suppression assay

Co-cultures were performed with a ratio of 2 B cells (50 000 cells/well)/2 responder T cells/1 Treg. Cells were activated with 1 µg/mL plate-bound anti-CD3 and 25 ng/mL IL-4 (R&D). 2 µg/mL neutralising anti-Fas antibody (ZB4) or a mouse IgG1 isotype control (Upstate Millipore) was added when specified. The % suppression was calculated as follows: 100×[(% B cell expression without Treg)−(% B cell expression with Treg)]/(% B cell expression without Treg).

CFSE staining

10^7 cells/mL responder T cells were resuspended in 1 µM carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen) diluted in phosphate buffered saline (Sigma) for 10 min, in dark. The reaction was stopped with prewarmed foetal calf serum (Biosera). The cells were washed and resuspended.

Apoptosis induction

B cells (50 000 cells/well) were cultured with irradiated CD40L-transfected Chinese hamster ovary (CD40L-CHO) cells at a 50:1 ratio with 25 ng/mL IL-4. Agonistic anti-Fas antibody (EOS9.1) or a mouse IgM isotype control (eBioscience) was added for the last 4 h of culture.

Flow cytometry

Staining was performed with the relevant antibodies according to manufacturer's instructions (see online supplementary text S1). For cytokine detection, 1 ng/mL phorbol-myristate acetate, 250 ng/mL ionomycin (Sigma-Aldrich) and 1 µL/mL GolgiPlug (BD-Biosciences) were added for the last 4 h of culture. Data were acquired on LSRII-flow-cytometer (Becton Dickinson) and analysed with FlowJo (Tree Star).

Apoptosis detection

eFluor450-AnnexinV and 7-aminoactinomycin (7-AAD) staining was performed in AnnexinV binding buffer (eBioscience). Apoptotic cells were defined as AnnexinV$^+$7-AAD$^+$ cells. The apoptosis induced by adding Treg (or agonistic Fas antibody) was defined as % increase in apoptosis and calculated as follows: [100×(% annexinV$^+$7-AAD$^+$B cells with Treg)/(% annexinV$^+$7-AAD$^+$B cells without Treg)]−100

ELISA

IgG and IgM were measured in supernatants by ELISA using a standard protocol (see online supplementary text S2).

Statistical analysis

Statistical analyses were performed with GraphPad/Prism software (San Diego, California, USA). Mann–Whitney test and Wilcoxon-signed-rank test were used, as specified.

RESULTS

Treg depletion increases B cell responses in healthy donors but not in RA patients

We first sought to determine if Treg depletion from PBMCs could affect B cell responses in healthy donors and RA patients. We studied B cell proliferation (Ki67) and used CD80/CD86 as activation markers. We also explored B cell cytokine secretion and antibody production. Treg depletion resulted in an increase of B cell proliferation (figure 1A) and activation (figure 1B,C) in healthy donors, whereas no significant change was observed in RA patients (figure 1A–C). Similar results were obtained for TNFα expression (figure 1D) and antibody production (figure

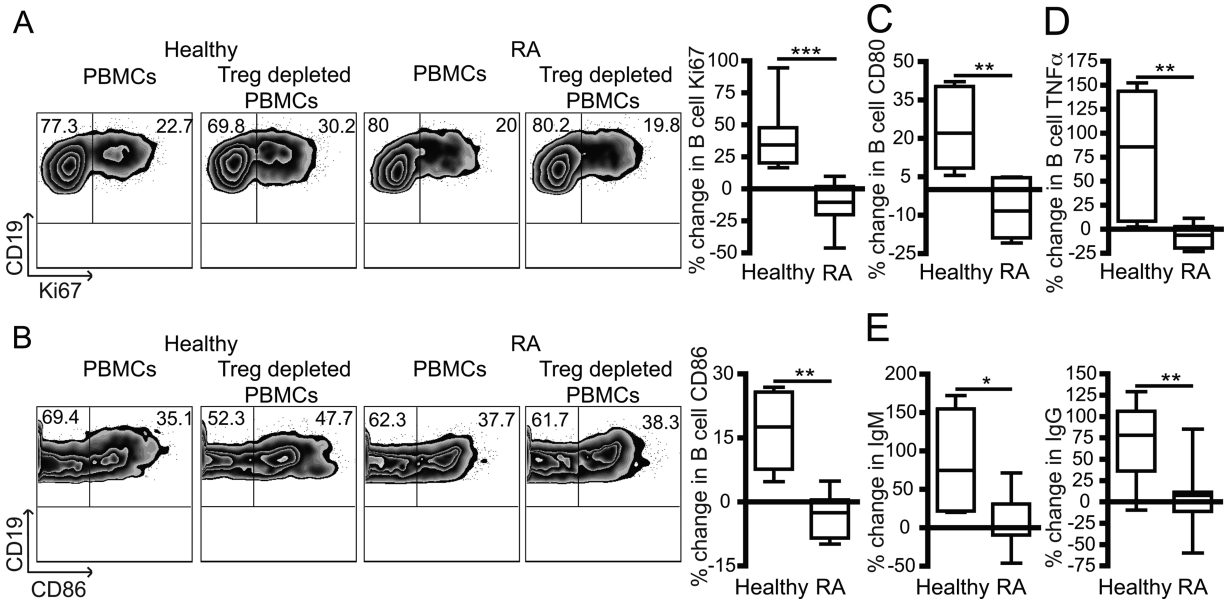


Figure 1 Treg depletion from peripheral blood mononuclear cells (PBMCs) increases B cell responses in healthy donors but not in rheumatoid arthritis (RA) patients. PBMCs or Treg-depleted PBMCs from healthy donors and RA patients were stimulated in vitro with soluble anti-CD3 for 3 (A–C) or 6 (D–E) days. Representative flow cytometry plots gated on CD19+ B cells showing expression of (A) Ki67 and (B) CD86. Cumulative data showing the % change in B cell (A) Ki67 (n=9), (B) CD86 (nHealthy=6; nRA=7), (C) CD80 (nHealthy=6; nRA=7) and (D) TNFα (nHealthy=6; nRA=8) after Treg depletion. (E) Supernatants collected at day 6 were assayed for IgM and IgG production by ELISA. Cumulative data showing the % change in IgM and IgG (n=9). The median, the 25th and 75th percentiles, and the minimum and maximum are represented. Mann–Whitney test was used to compare healthy donors and RA patients (*p<0.05, **p<0.01 and ***p<0.001).

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1E). These results suggest that Treg can limit a wide range of B cell responses in healthy donors but not in RA patients.

B cell suppression by Treg is defective in RA patients

To confirm Treg capacity to control B cell responses in healthy donors and their putative defects in RA, we then performed suppression assays by co-culturing B and responder T cells with or without Treg. Treg efficiently suppressed B cell responses in healthy donors (figure 2A–F). In contrast, Treg in RA were twofold to threefold less effective in controlling B cell proliferation and CD80/CD86 expression (figure 2A–C). Additionally, their capacity to reduce B cell TNFα and IL-6 production was significantly compromised (figure 2D,E). Both IgM and IgG production were minimally reduced by Treg in RA patients in contrast to the >80% suppression observed for healthy donors (figure 2F).

Treg can control effector cells by inducing their apoptosis.[16] We therefore determined if Treg could specifically increase B cell apoptosis in healthy donors and RA patients. We observed a substantial increase in B cell apoptosis in healthy donors when Treg were added to B cells in the presence of responder T cells but not in RA patients (figure 2G). Taken together, these results highlight the impaired ability of Treg to limit B cell responses in RA.

Dual defects coexist in Treg and B cells from RA patients

To further dissect Treg failure to restrain B cells in RA, we explored whether these defects could be ascribed to intrinsic abnormalities in Treg function and/or resistance to suppression of B (or responder T) cells. Thus, we performed suppression assays with B cells, responder T cells and Treg from RA patients and healthy donors, mixing target and regulatory cells using a three-way 'crossover' design.

We first tested RA Treg capacity to suppress healthy B cells (in co-culture with autologous healthy responder T cells) compared with healthy Treg. RA Treg were significantly less efficient at suppressing healthy B cell proliferation than healthy Treg (figure 3A). Similar results were obtained for suppression of B cell activation (figure 3B). RA Treg were also impaired in their ability to induce apoptosis in healthy B cells (figure 3C). To ascertain if the defects observed were due to the disease, and not an allogeneic response, we mixed healthy B and responder T cells with autologous or allogeneic Treg. No significant differences were observed (see online supplementary figure S2A). Therefore, RA Treg are intrinsically defective in suppressing B cells in RA, which is consistent with our previous data.[6] However, the suppression obtained when RA Treg were cultured with healthy B cells was only significantly greater compared with RA Treg and RA B cells with respect to suppression of B cell CD86 expression (figure 3B), and a trend towards greater suppression of B cell proliferation as assayed by Ki67 expression ($p=0.084$) (figure 3A). These data suggest that B cell resistance to suppression may contribute to the failure of RA Treg to control B cell function in RA.

We therefore performed the converse experiments and tested whether RA B cells were resistant to Treg suppression. We compared healthy and RA B cell suppression by the same healthy Treg population, in co-culture with healthy responder T cells. Healthy Treg were less efficient at suppressing RA B cell proliferation (figure 3A) and activation (figure 3B) compared with healthy B cells. Similarly, healthy Treg were much less competent at inducing apoptosis in RA B cells compared with healthy B cells (figure 3C). Indeed, there was no difference in the degree of suppression of RA B cells when healthy Treg replaced RA Treg. Importantly, these defects were observed irrespective of the provenance of the responder T cells (see online supplementary figure S2B,C), suggesting that responder T cells are not resistant to suppression, consistent with our previous findings.[6] These data indicate that RA B cells are resistant to Treg suppression and tend to suggest that the main contributing factor to the lack of control of RA B cells by Treg is the former's resistance to suppression.

Differential Fas expression in RA and healthy B cells

As Fas-mediated apoptosis is critical for the elimination of autoreactive B cells,[17 18] we investigated Fas expression on B cells. Fas is induced in B cells upon activation.[18] Following in vitro activation in the presence of responder T cells, Fas was strongly expressed in B cells from healthy donors (figure 4A,B). However, Fas expression was twofold lower in RA B cells compared with their healthy counterparts (figure 4B). Upon addition of Treg, we observed a 50% reduction in Fas expression on healthy B cells in contrast to a 25% reduction on RA B cells (figure 4A,C). As Fas internalisation is a prerequisite for signal transduction leading to apoptosis,[19 20] the decrease of Fas at the surface of healthy B cells may indicate its engulfment following addition of Treg to the co-cultures. To provide further support for this hypothesis, we examined downstream elements of Fas pathway. We studied the activation of a major effector Caspase (Caspase3) and the cleavage of the poly ADP ribose polymerase 1 (PARP1), a key substrate of the Caspase family, whose integrity is vital within the cell.[17] We found a 20% increase of the active form of Caspase3 (figure 4D) and a 30% increase of the cleaved form of PARP1 (figure 4E) in B cells from healthy donors when exposed to Treg, while no change was observed for B cells from RA patients (figure 4D,E). These data indicate that Treg can trigger B cell Fas-mediated apoptosis in healthy individuals but not in RA patients.

B cell suppression by Treg is dependent upon Fas in healthy donors

To investigate whether Fas was required for B cell suppression by Treg in healthy individuals, we set up suppression assays as described earlier with a blocking anti-Fas antibody. Fas blockade significantly reduced Treg suppression of B cell proliferation (figure 5A), activation (figure 5B) and production of TNFα (figure 5C), IgM and IgG (figure 5D) in healthy donors. Similarly, the capacity of Treg to increase B cell apoptosis was impaired by the addition of the blocking antibody (figure 5E). These data demonstrate that Fas engagement on B cells is required for effective Treg control of B cell responses in healthy donors. Interestingly, B cells and T responder cells but not Treg expressed FasL (see online supplementary figure S3).

RA B cells are resistant to Fas-mediated apoptosis

Finally, we wanted to ascertain if RA B cells were resistant to Fas-mediated apoptosis. Activated responder T cells are sensitive to Fas-mediated apoptosis.[17] To assess B cell resistance to Fas-mediated apoptosis, independently of responder T cells, purified B cells were co-cultured with CD40L-CHO cells. In this system, B cells were activated by CD40 L, mimicking responder T cell activation. Fas-mediated apoptosis was induced using agonistic anti-Fas antibody. In the presence of anti-Fas antibody, apoptosis increased in healthy B cells whereas RA B cells were intrinsically resistant to Fas-mediated apoptosis (figure 6A). Similarly, no elevation of active Caspase3 and cleaved PARP1 occurred in B cells from RA patients in the

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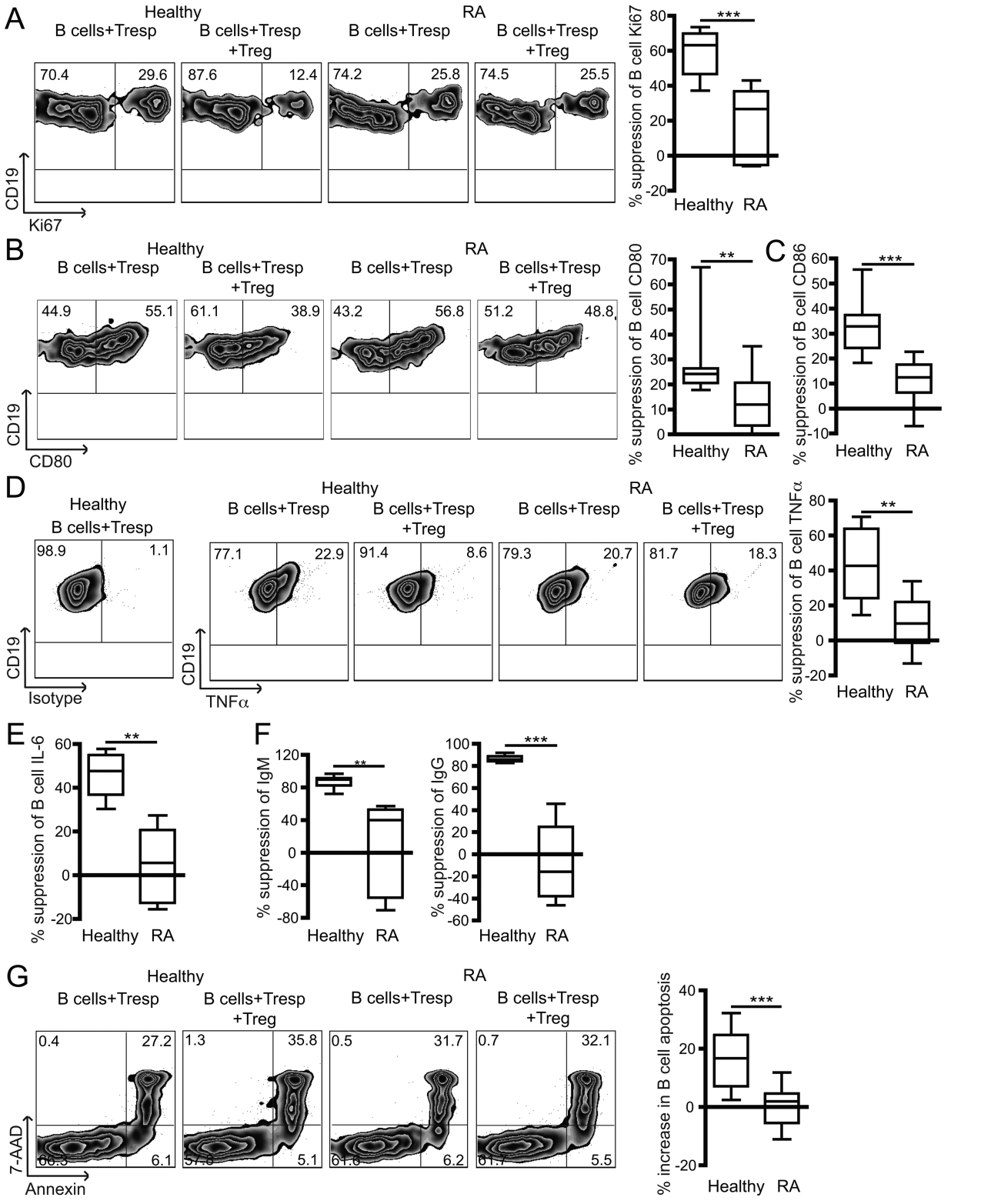


Figure 2 B cell suppression by Treg is defective in rheumatoid arthritis (RA) patients. B cell suppression assays were set up in healthy donors and RA patients. B cells and responder T cells (Tresp) were co-cultured with or without autologous Treg (ratio used 2:2:1, respectively) and stimulated for 4 days with plate-bound anti-CD3 and IL-4. Representative flow cytometry plots showing expression of B cell (A) Ki67, (B) CD80 and (D) TNFα. Cumulative data showing the % suppression by Treg of B cell (A) Ki67 (n=14), (B) CD80 (n=12), (C) CD86 (n=12), (D) TNFα (n=8) and (E) IL-6 (n=5). Cytokine expression was assessed relative to isotype-matched control antibody. (F) Supernatants collected at day 7 were assayed for IgM and IgG production by ELISA. Cumulative data showing the % suppression of IgM and IgG secretion (nHealthy=8; nRA=10). (G) Representative flow cytometry plots of B cell apoptosis in the presence or absence of Treg and cumulative data showing the % increase in B cell apoptosis (nHealthy=11; nRA=13). The median, the 25th and 75th percentiles, and the minimum and maximum are represented. Mann–Whitney test was used (**p<0.01 and ***p<0.001).

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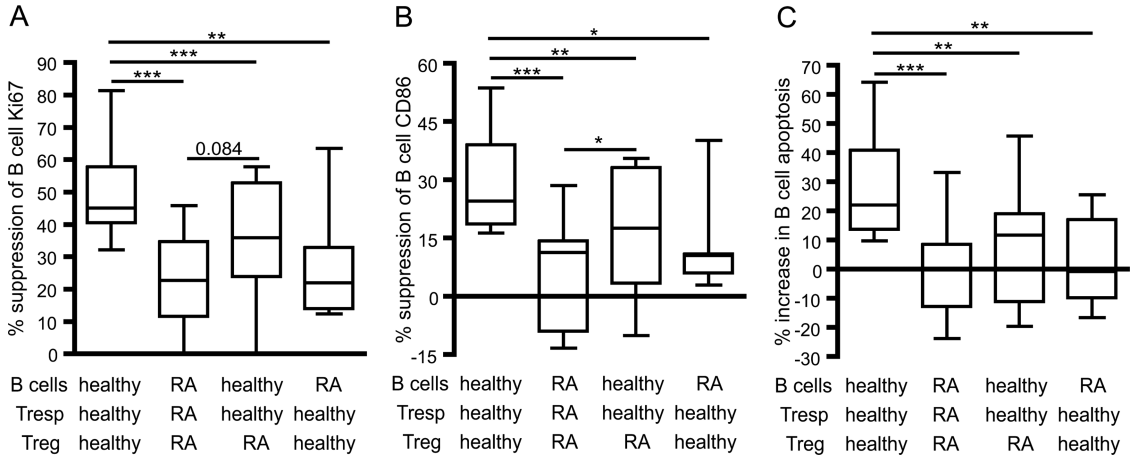


Figure 3 Dual defects coexist in Treg and B cells from rheumatoid arthritis (RA) patients. Suppression assays were performed as described in figure 2. B cells were co-cultured with autologous or allogeneic responder T cells (Tresp) in the presence or absence of autologous or allogeneic Treg. Cumulative data showing the % suppression by Treg of B cell (A) Ki67 (n=9–12) and (B) CD86 (n=7–10). (C) Cumulative data showing the % increase in B cell apoptosis (n=9–12). The median, the 25th and 75th percentiles, and the minimum and maximum are represented. Mann–Whitney test was used to compare healthy donors versus RA patients and Wilcoxon test was used for crossover experiments (*p<0.05, **p<0.01 and ***p<0.001).

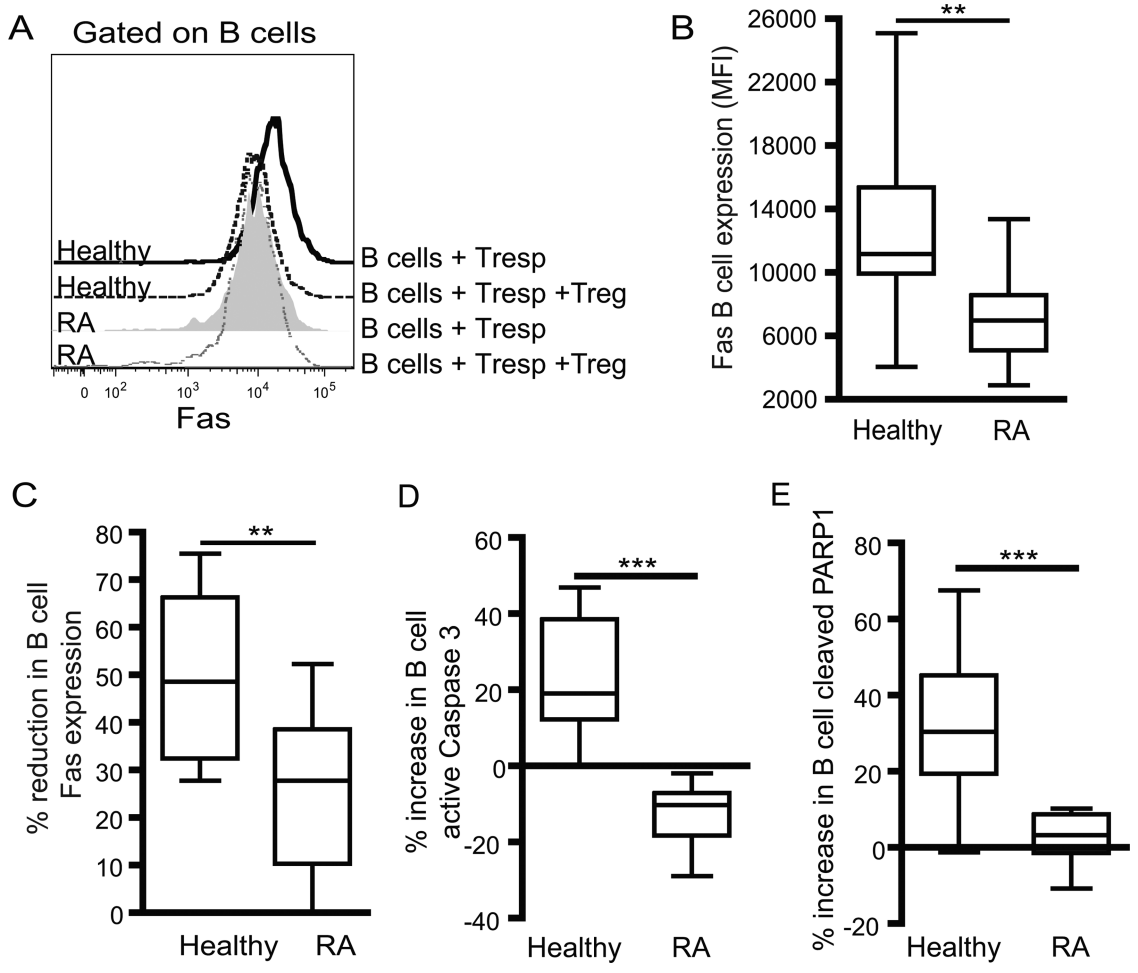


Figure 4 Differential Fas expression is found in rheumatoid arthritis (RA) and healthy B cells. Suppression assays were performed as described in figure 2. (A) Representative histogram showing expression of Fas on B cells from co-cultures of B cells and responder T cells with or without Treg. (B) Cumulative data comparing Fas expression on B cells when cultured with responder T cells (nHealthy=14; nRA=10). (C) Cumulative data showing the % reduction in B cell Fas expression following addition of Treg to the co-culture (nHealthy=14; nRA=10). (D) Cumulative data showing the % increase in B-cell-activated Caspase3 following addition of Treg to the co-culture (n=8). (E) Cumulative data showing the % increase in B-cell-cleaved poly ADP ribose polymerase 1 (PARP1) following addition of Treg to the co-culture (n=10). The median, the 25th and 75th percentiles, and the minimum and maximum are represented. Mann–Whitney test was used (**p<0.01 and ***p<0.001).

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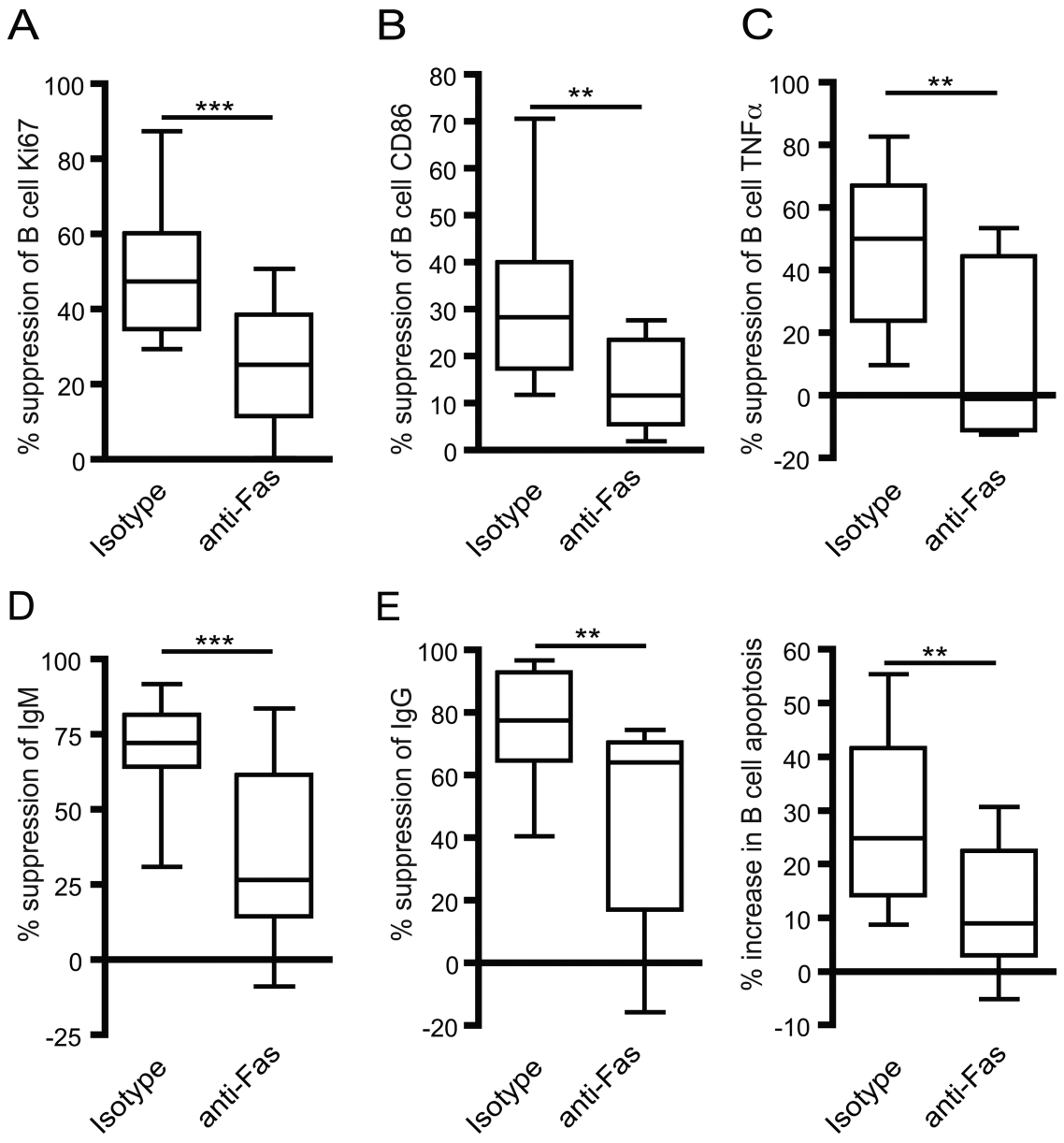


Figure 5 B cell suppression by Treg in healthy donors is dependent upon Fas. Suppression assays were performed on healthy donors as described in figure 2. 2 μg/mL of blocking anti-Fas antibody or an isotype control was added to suppression assays and B cell responses were assessed by flow cytometry and ELISA. Cumulative data showing the % suppression by Treg of B cell expression of (A) Ki67 (n=14), (B) CD86 (n=9), (C) TNFα (n=8) and secretion of (D) IgM (n=12) and IgG (n=5). (E) Cumulative data showing the % increase in B cell apoptosis (n=9). The median, the 25th and 75th percentiles, and the minimum and maximum are represented. Wilcoxon test was used (**p<0.01 and ***p<0.001).

presence of the agonistic antibody, whereas increases were observed for healthy donors (figure 6B,C). These data demonstrate RA B cell resistance to Fas-mediated apoptosis.

DISCUSSION

In this study, we have shown that Treg limit the multifaceted B cell response in healthy donors but not in RA patients. Defective regulation in RA is due not only to intrinsic defects in Treg but also as a result of B cell resistance to suppression. Indeed, our data suggest that the latter is primarily responsible for this failure in regulation. We tested B cell suppression by Treg in the presence of responder T cells and established that the last were not resistant to suppression in RA patients in keeping with our previous data[9] and others.[7] Both the suppression of B cells by Treg in healthy subjects and B cell resistance to regulation in RA patients was linked to Fas. This pathway, which is known to be crucial for immune homeostasis,[17] has not been previously implicated in the regulation of B cells by Treg in humans, nor has a defect been described in Fas/FasL interactions in the context of Treg control of an autoimmune disease.

Treg exert their suppressive activity through different mechanisms, resulting in anergy or death of various target cells.[5 16] Murine studies have revealed that Treg can restrain B cell responses both indirectly (by acting on responder T cells) and directly.[21–24] Treg control of B cells in humans has been less well characterised. One group showed that Treg from tonsils can inhibit B cell function both in the presence of responder T cells[25] and directly.[26] In agreement with these studies, we showed that Treg limit B cell proliferation, activation, cytokine

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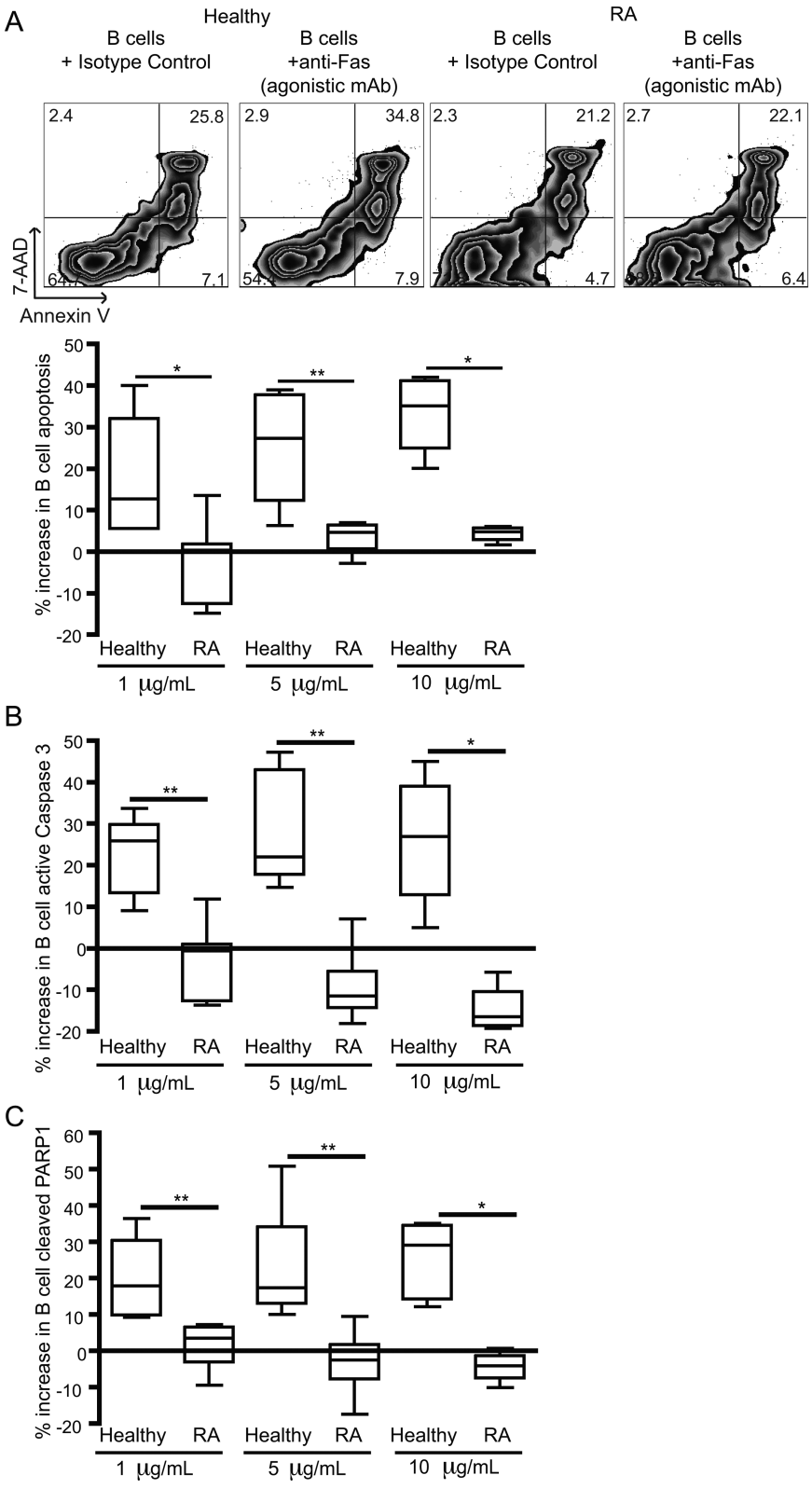


Figure 6 Rheumatoid arthritis (RA) B cells are intrinsically resistant to Fas-mediated apoptosis. B cells isolated from healthy donors and RA patients were stimulated with CD40L-CHO cells and IL-4 for 3 days. For the last 4 h of culture, agonistic anti-Fas antibody or an isotype control was added. (A) Representative flow cytometry plots showing B cells undergoing apoptosis due to addition of 10 µg/mL agonistic anti-Fas antibody or isotype control. Cumulative data showing the % increase in B cell apoptosis using 1, 5 and 10 µg/mL agonistic anti-Fas antibody compared with isotype control (nHealthy=5–11; nRA=5–13). (B) Cumulative data showing the % increase in B-cell-activated Caspase3 using 1, 5 and 10 µg/mL agonistic anti-Fas antibody compared with isotype control (nHealthy=5–6; nRA=5–7). (C) Cumulative data showing the % increase in B-cell-cleaved poly ADP ribose polymerase 1 (PARP1) using 1, 5 and 10 µg/mL agonistic anti-Fas antibody compared with isotype control (nHealthy=5–6; nRA=5–7). The median, the 25th and 75th percentiles, and the minimum and maximum are represented. Mann–Whitney test was used (*p<0.05, **p<0.01).

and antibody production and induce apoptosis in healthy donors.

Several mechanisms of B cell control by Treg have been proposed in mice, including TGFβ, PD1 and perforin/granzyme-pathway.[21–24 27] In humans, TGFβ in association with CTL-A4[25 26] or GITR/IL-10[28] has been implicated. Our study highlights Fas involvement in Treg suppression of B cells. Fas-mediated apoptosis of B cells has been suggested using a Treg murine cell line in vitro.[29] However, Fas does not appear to be crucial for direct suppression of B cells in mice models.[21 22] Consistent with these findings, we detected very low FasL expression on Treg, which is likely secondary to its direct suppression by Foxp3.[30] Rather our data suggest that Treg act on responder T cells through a separate pathway that has not been defined. We thus propose a sequence of events triggered by Treg and mediated by responder T cells whereby B cells are

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restrained through induction of Fas-mediated apoptosis. Consistent with this hypothesis, a mouse study revealed that Treg did not express Fas or FasL but engaged the Fas pathway to control dendritic cells.[31] Alternatively, it is possible that Fas/FasL on B cells interact inducing autocrine and paracrine suicide, as previously described.[32] Although induction of B cell apoptosis may explain the full range of B cell responses restrained by Treg, Fas can mediate non-apoptotic responses,[33] which may account for some of the suppressive effects.

B cell Fas expression increases upon stimulation, and activated B cells are sensitive to Fas-mediated apoptosis,[18] which is in agreement with our analysis of healthy B cells. Conversely, activated RA B cells were less sensitive to Fas-mediated apoptosis. Not only was Fas upregulation following activation diminished for RA B cells compared with their healthy counterparts, but the Treg-driven reduction in Fas surface expression was also markedly less. In fact, the level of Fas expressed at the cell surface may influence the sensitivity/resistance to FasL.[18 34 35] Sensitivity to Fas-mediated apoptosis has been linked to its level of expression in a murine model of SLE.[36] Therefore, the reduced Fas expression in RA B cells could account for their resistance to agonistic anti-Fas antibody and to Treg control. Although it is possible that Fas is less expressed following Treg encounter, the reduction in Fas expression on healthy B cells may reflect its internalisation through an endosomal pathway following its ligation with FasL.[37 38] The associated increased activation of the Caspase3 and cleavage of PARP1, both of which lie downstream of Fas signalling, in healthy donors but not in RA patients tend to support this hypothesis. Fas internalisation is a prerequisite to apoptosis, whereas engagement of Fas without internalisation results in activation of non-apoptotic pathways.[19 20] Our data are also consistent with a reduction in the efficiency of Fas internalisation/signalling in RA B cells. Of relevance, resistance to Fas-mediated apoptosis has been associated with reduced endocytosis.[20] Taken together, reduced Fas expression and internalisation/signalling could account for both RA B cell resistance to Fas-mediated apoptosis induced with an agonistic antibody and the failure of Treg suppression of B cells in RA.

Mice that are Fas-sufficient except for the B cell lineage exhibit both B and T cell hyperproliferation and autoimmune disease,[39] indicating the pivotal B cell Fas-dependent interplay between these two populations to maintain tolerance. Restricting B cell responses is dependent upon FasL expression by responder T cells.[40] Thus, Fas-expressing B cells interacting with FasL on responder T cells provide a target for Treg to govern humoral immune responses. This is consistent with previous data indicating that Treg control of B cells is entirely dependent upon the presence of responder T cells.[12] Our data also show that the provenance of the responder T cells (healthy or RA) did not alter the outcome of suppression. Thus although responder T cells are an important intermediary in governing B cell suppression, their function with respect to B cell control does not appear to be altered in RA. In healthy individuals, FasL expression is reduced on responder T cells but not on B cells in the presence of Treg. Although FasL could simply be less expressed following Treg encounter, this decrease could reflect its interaction with Fas on B cells triggering internalisation. FasL could be shed by mettalloproteases[41] or released within bioactive exosomes[42 43] and has been found inside target cells.[38 44] Therefore, Treg could indirectly trigger Fas-mediated apoptosis on B cells via FasL expressed on responder T cells or on B cells themselves, although the underlying mechanism remains unclear. Insight may come from our observations that RA Treg have reduced CTLA-4 expression.[8] It has been shown that CLTA-4 can contribute to Treg control of B cells[25 26] and modulate FasL expression.[45 46]

Collectively our data prompt a re-evaluation of therapeutic strategies for autoimmune diseases such as RA, which focus on harnessing Treg.[47 48] Although Treg are impaired in RA, correcting this defect alone would be insufficient to limit B cell responses due to the latter's resistance to control. The only currently available strategy that specifically targets RA B cells is to deplete them.[3] Developing a more specific therapeutic that targets the Fas pathway in B cells could enhance their susceptibility to regulation and would complement efforts to boost Treg function to treat RA patients.

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Contributors LR and MRE conducted the research planning and designed research. LR, K-MC and CME performed the experiments. LR, K-MC, CME and MRE analysed data. LR and MRE wrote the paper.

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Competing interests None.

Patient consent Obtained.

Ethics approval All clinical investigations have been conducted according to the Declaration of Helsinki principles. The University College London Hospital Ethics Committee approved this study.

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