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Abatacept Limits Breach of Self-Tolerance in a Murine Model of Arthritis via Effects on the Generation of T Follicular Helper Cells

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Abatacept modulates CD28-mediated T cell costimulation and is efficacious in the treatment of rheumatoid arthritis (RA). Its mechanism of action has not been fully elucidated but will likely reveal critical pathologic pathways in RA. We show that abatacept substantially modulated Ag-specific T and B cell responses in vivo. Ag-specific T cell proliferation was reduced, and the acquisition of an activated phenotype, characterized by upregulation of CD69, OX40, ICOS, and programmed death-1 and downregulation of CD62L, was suppressed. Furthermore, abatacept suppressed the production of inflammatory cytokines, such as IFN-γ and IL-17. These effects were associated with a failure of Ag-specific T cells to acquire the CXCR5+ICOS+ T follicular helper cell phenotype. This, in turn, led to a failure of these cells to enter B cell follicles, resulting in reduced specific Ab responses, despite normal B cell clonal expansion. To test the pathologic significance of this, we used a novel model of RA associated with breach of self-tolerance to self-Ag and demonstrated that abatacept prevented the emergence of self-reactivity. Thus, CD28-dependent signaling is required for optimal T follicular helper cell maturation and expansion, and its inhibition prevents loss of self-tolerance in a model of articular pathology. Thus, we provide a novel mode of action for abatacept with profound implications for its potential usefulness in early inflammatory arthropathies associated with autoantibody expression.  The Journal of Immunology, 2010, 185: 000–000.

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial inflammation with concomitant cartilage and bone destruction. A critical role for adaptive immunity in disease pathogenesis is supported by the presence of activated T cells in the synovial lesion, by long-established association with HLADR04, and by recent genome-wide scanning studies implicating ptpn22, cd40, citla4, and cd28 (1–3). However, definitive proof of concept for adaptive immune involvement resides in the proven efficacy of therapeutics, such as rituximab and abatacept, targeting B and T lymphocytes, respectively (4, 5). Despite these therapeutic successes, considerable unmet clinical need remains in RA, because partial or nonresponders are common, and remission is rarely achieved or sustained. Moreover, combinatorial approaches using existing biologics have been common, and remission is rarely achieved or sustained. Therefore, we evaluated the mechanism whereby abatacept, as a soluble fusion protein consisting of the extracellular domain of CTLA-4 and the Fc portion of human IgG1, can compete with CD28 for CD80/CD86 binding and inhibits its in vitro T cell proliferation in human MLRs (12). CTLA-4lg was also demonstrated to ameliorate autoimmunity in vivo during collagen-induced arthritis, experimental autoimmune encephalomyelitis, and systemic lupus erythematosus, and it can prolong allograft and xenograft survival (13–17). Few mechanistic data are available on exactly how, where, or when CTLA-4Ig exerts its effects directly on T cells in vivo. Furthermore, its effects on T-dependent B cell responses in vivo are poorly characterized. Elucidating such effects should allow optimization of its therapeutic application and yield insight about novel therapeutic modalities. Therefore, we evaluated the mechanism whereby abatacept, a soluble fusion protein consisting of the extracellular domain of CTLA-4 and a modified Fc portion of human IgG1 that eliminates Ab-dependent cell-mediated cytotoxicity and complement fixation activity, affects pathogenesis of RA, which, in turn, is dependent, in part, upon properly understanding the mode of action of effective agents.

During the induction of a T cell response, TCR-mediated signals without costimulation are insufficient for full T cell activation. The best-characterized costimulatory moiety is CD28, expressed by all CD4+ T cells and some CD8+ T cells (7). CD28 binds CD80 and CD86 on APCs with low affinity and is obligatory for full T cell activation, as well as IL-2 production and proliferation (8, 9). CTLA-4 is structurally homologous to CD28, binds to CD80/CD86 on APCs with higher affinity than CD28, and, thereby, acts as a negative regulator of CD28-mediated T cell costimulation (10). Although low levels of intracellular CTLA-4 reside in naive T cells, surface CTLA-4 expression is restricted to effector T cells and T regulatory cells (11). CTLA-4lg, a soluble fusion protein consisting of the extracellular domain of CTLA-4 and the Fc portion of human IgG1, can compete with CD28 for CD80/CD86 binding and inhibits its in vitro T cell proliferation in human MLRs (12). CTLA-4lg was also demonstrated to ameliorate autoimmunity in vivo during collagen-induced arthritis, experimental autoimmune encephalomyelitis, and systemic lupus erythematosus, and it can prolong allograft and xenograft survival (13–17). Few mechanistic data are available beyond observation of reduced alloreactive T cell clonal expansion due to growth factor withdrawal (18) and reduced murine Ab responses (19).

Thus, despite extensive clinical application, remarkably limited information is available on exactly how, where, or when CTLA-4lg exerts its effects directly on T cells in vivo. Furthermore, its effects on T-dependent B cell responses in vivo are poorly characterized. Elucidating such effects should allow optimization of its therapeutic application and yield insight about novel therapeutic modalities. Therefore, we evaluated the mechanism whereby abatacept, a soluble fusion protein consisting of the extracellular domain of CTLA-4 and a modified Fc portion of human IgG1 that eliminates Ab-dependent cell-mediated cytotoxicity and complement fixation activity, affects
Ag-specific lymphocyte behavior in vivo. We used transgenic (Tg) T cell and B cell co-transfer models to dissect the effects of abatacept on specific T cell responses and T-dependent B cell responses using trackable lymphocyte populations. Abatacept profoundly suppressed Ag-specific T cell proliferation and phenotypic activation. Furthermore, the production of inflammatory mediators, such as IFN-γ, IL-17, and MIP-1α, was reduced. Crucially, these effects were associated with a failure of lymph node (LN) Ag-specific T cells to acquire a CXCXR5+ICOS+ T follicular helper (TFH) cell phenotype. This led to a failure of TFH cell migration to B cell follicles, resulting in reduced specific Ab responses, despite normal B cell clonal expansion. The clinical relevance of these data was clearly demonstrated in a novel model of RA in which abatacept prevented breach of self-tolerance, reducing anti-self Ab responses.

**Materials and Methods**

**Mice**

IgHb BALB/c (H-2d, IgMb) mice were bred in-house (Central Research Facilities, University of Glasgow). Mice homozygous for the chicken OVA peptide323-339/I-Ad-specific DO11.10 TCR transgenes (detected using the clonotypic mAb KJ1.26) on the BALB/c background were used as donors, as were mice heterozygous for the anti-hen egg lysozyme (HEL) IgM and IgD transgenes on the BALB/c background (MD4). BALB/c or IgHb mice 6–12 wk old were used as recipients. All animals were specified pathogen-free and were maintained under standard animal house conditions in accordance with local and home office regulations.

**Reagents**

Abatacept was provided by Bristol-Myers Squibb. Chi L6 is a chimeric fusion protein consisting of the V region of murine L6 Ag, combined with a human IgG1 C region; it was used as a control fusion protein (control Ig) in these studies. Abatacept and control Ig were injected i.p. at a dose of 10 mg/kg.

**Preparation of cell suspensions for adoptive transfer**

Peripheral LNs (pLNs) and spleens from MD4 BALB/c and DO11.10 BALB/c mice were pooled and prepared as described previously (20). Cells were labeled with 8.5 μM CFSE in HBSS for 10 min at 37°C, as described previously (20), before cell suspensions containing 1 × 10⁶ CFSE-labeled Tg T cells and 1 × 10⁶ unlabeled Tg T or B cells were injected i.v. into nonirradiated, age-matched IgHb BALB/c recipients, as previously described (20). Alternatively, 5 × 10⁶ cells were labeled with 5 μM Cell Tracker Red (CMTX) in CO₂-independent media (Invitrogen, Paisley, Scotland, U.K.) for 40 min at 37°C for multiphoton imaging.

**Adoptive transfer of OVA-specific Th1 cells and arthritis model**

Arthritis was induced in BALB/c recipients by i.v. transfer of 2 × 10⁶ in vitro, Th1-polarized, KJ1.26+ T cells, followed 1 d later by restimulation in vivo with 100 μg OVA in CFA in the scruff of the neck; 10 d later, 100 μg heat-aggregated OVA (HAO) alone was given by footpad injection. Mice were monitored for development of arthritis, as described previously (21).

**Immunofluorescence analysis of tissue sections and confocal microscopy**

Ag-specific T cell localization in the LN was determined by immunofluorescence as described previously (20), using B220-FITC to stain B cell areas, anti-CD4 Alexa Fluor 450 for T cell areas, and biotinylated KJ1.26 to detect OVA-specific cells and was visualized using streptavidin-Alexa Fluor 647 (Molecular Probes, Eugene, OR). All photographs were taken at ×20 or ×40 magnification.

**Flow cytometry**

Aliquots containing 1 × 10⁶ cells were incubated in 200 μl FACS buffer (PBS/2% FCS) for 15 min at 4°C with purified anti-mouse CD16/CD32 (BD Biosciences, San Jose, CA), washed, and incubated with the appropriate fluorochrome-conjugated or biotinylated primary Abs for 30 min at 4°C. The cells were washed, resuspended in FACS buffer and, where appropriate, biotinylated Abs were detected by fluorochrome-conjugated streptavidin for an additional 15 min. Cells were then analyzed on a FACSCanto Flow Cytometer (BD Biosciences) using FlowJo software.

**In vitro Ag-specific cytokine production**

Cytokine levels were determined in cultures of total draining LN cells stimulated with 5 μM OVA323–339 peptide (Sigma-Aldrich, St. Louis, MO) for 48 h. Cells were cultured at 2.5 × 10⁶ cells/200 μl in round-bottom 96-well plates. Supernatants were assayed for levels of fibroblast growth factor; GM-CSF; IFN-γ; IL-1α/β, 2, 4, 5, 6, -10, -12 (p40/p70), -13, and -17; CXCL10; CXCL1; MCP-1; CXCL9; TNF-1, and vascular endothelial growth factor by Luminex (In vitro) and analyzed using a Luminex XMAP system, according to the manufacturer’s instructions.

**In vitro restimulation assay**

pLN cells were cultured with medium or 1 mg/ml OVA. Proliferation was analyzed at 96 h by flow-cytometric staining for EdU (5-ethyl-2'-deoxyuridine) incorporation (Invitrogen).

**In vivo challenge**

One day after adoptive transfer of naïve T cells, recipient BALB/c mice were challenged in the footpad with 100 μg OVA in CFA or CFA only as an adjuvant control (or PBS, because no difference in KJ1.26 expansion between PBS and CFA challenge was seen). Chicken OVA (fraction V) was obtained from Sigma-Aldrich, and HEL was obtained from Biozyme (Gwent, U.K.). Conjugated OVA-HEL was prepared as previously described (20). Animals were injected s.c. with 150 μg chicken OVA-HEL or 100 μg OVA, both in CFA (Sigma-Aldrich).

Quantification of follicular migration of Ag-specific T cells

The proportion of OVA-specific (KJ1.26+) cells was quantified using Volocity software. The localization of Tg T cells and B cell follicles was plotted. Using these tissue maps, the number of Tg T cells in defined gates was calculated for three gates for the total section and three B cell follicle gates per section. Data are plotted as the mean proportion of transgenic Tg T cells (KJ1.26+) within the follicle (B220⁺), calculated by dividing the number of Tg T cells in the B220⁺ follicle by the total number of KJ1.26⁺ cells within the total field of view. Data shown are the mean of triplicate readings from three mice per group.

Ab ELISA

Anti-OVA, anti-HEL, and anti-collagen Abs were detected as previously described (20, 21).

**Multiphoton microscopy**

To image cellular behavior in LNs, excised LNs were transferred into CO₂-independent media at room temperature. The LN was bound with veterinary glue (Vetbond, 3M, St. Paul, MN) onto a plastic coverslip that was then adhered with grease to the bottom of the imaging chamber continuously supplied with warmed (36.5°C) and gassed (95% O₂ and 5% CO₂) RPMI 1640 before and throughout the period of microscopy and was imaged, as previously described (22).

**Statistical analysis**

Results are shown as mean ± 1 SD, unless stated otherwise, and groups were compared using the Student two-tailed unpaired t test. Two-way ANOVAs were used to compare Ab levels.

**Results**

Abatacept modulated phenotypic and functional aspects of Ag-specific T cell activation

Abatacept inhibits T cell proliferation in vitro in human MLRs and in vivo in alloresponses (12, 18). We first assessed whether this could be reproduced in our Ag-specific model system in vivo. We measured division of CFSE-labeled, DO11.10 OVA-specific T cells transferred into naive BALB/c recipients that were then immunized s.c. with OVA/CFA. Single administration of abatacept immediately after immunization significantly decreased the proportion and total number of OVA-specific T cells in the draining LN (dLN) 3 d postimmunization (Fig. 1A, data not shown). Comensurate with this, Ag-specific T cell proliferation was reduced, represented by an increased proportion of CD4⁺KJ1.26⁺ cells that had undergone fewer than four divisions (Fig. 1B, 1C).

Next, we assessed the ability of abatacept to modulate the activation of OVA-specific T cells in vivo. As before, naive BALB/c...
mice received 1 × 10⁶ DO11.10 T cells i.v. and were immunized with OVA/CFA in the footpad 24 h later. Three days later, the draining popliteal LN was harvested, and OVA-specific T cells were assessed for the expression of activation markers and markers associated with tolerance and/or regulatory T cells. Abatacept significantly reduced Ag-induced downregulation of CD62L on OVA-specific T cells (Fig. 2A), and the upregulation of OX40 by Ag-specific T cells (Fig. 2B). Strikingly, abatacept prevented the Ag-induced expression of ICOS and programmed death-1 (PD-1) (Fig. 2C, 2D), which are coexpressed by CXCR5⁺ TFH cells (23). The expression of CD28 and CD25 3 d postimmunization was not altered by treatment with abatacept (Fig. 2E, data not shown). Finally, CD69 expression by Tg T cells 1 d postimmunization was also significantly reduced by treatment with abatacept (data not shown).

The ability of abatacept to alter Ag-specific T cell functionality was assessed following transfer of DO11.10 T cells and OVA/CFA immunization. On day 3 postimmunization, total dLN cells were cultured in vitro with or without OVA peptide (323–339) for 48 h, and the supernatants were analyzed by Luminex. Production of inflammatory mediators was only detectable when OVA peptide was present in vitro and then only from cultures derived from animals receiving OVA/CFA in vivo (Fig. 2F–I). Abatacept significantly decreased synthesis of the inflammatory mediators IFN-γ, IL-17, MIP-1α, and IL-13 compared with control IgG-treated animals.

Abatacept failed to alter T cell velocity or meandering in dLNs
We and other investigators demonstrated previously that formation of dendritic cell (DC)–T cell clusters of smaller sizes and interactions of shorter duration fail to drive fully productive T cell responses (22, 24). Together with the postulated mode of action of abatacept, this prompted us to use multiphoton microscopy to examine whether abatacept affected the migration of specific T cells in intact LNs ex vivo. CMTPX-labeled DO11.10 T cells were adoptively transferred into BALB/c mice that were subsequently immunized s.c. with OVA/CFA and treated with control IgG or abatacept. To directly compare T cell behavior in immunized mice following treatment with control IgG or abatacept, the volume and location of each cell were measured in 15 planes per three-dimensional stack collected at 1.95-μm intervals between each plane, progressing deeper into the LN from an initial depth of 100 μm below the surface. Twenty-four hours after immunization, Ag-specific T cells had similar velocities and meandering indices (a measurement of directionality of cell movement) in the presence of abatacept compared with control IgG (Fig. 3A, 3B, Supplemental Videos 1, 2). Previous studies by ourselves and other investigators showed that DCs migrate to the LN within 24 h of immunization; this is when the interaction with DCs that determines the outcome for the T cell is taking place (22). To determine whether abatacept affected T cell interactions with APCs, we analyzed the proportion of T cells in clusters, the number of cells in clusters, and the number of cells per cluster, as previously described (22). Although a trend for a reduced proportion of T cells in clusters and the number of cells in clusters was apparent in the presence of abatacept, it was not significant (Fig. 3C, 3D). In addition, there was no difference in the number of T cells per cluster after abatacept treatment (Fig. 3E).

Abatacept suppressed acquisition of TFH cell phenotype and follicular migration of Ag-specific T cells
Although abatacept did not alter T cell velocity within the dLNs, the reduced expression of markers associated with a TFH cell phenotype prompted us to investigate whether abatacept affected the localization of Ag-specific T cells within LNs in situ. BALB/c mice were adoptively transferred with DO11.10 T cells and immunized with OVA/CFA, as before. Three days later, the dLN was assessed for
the localization of Ag-specific T cells, as shown previously (25).
Following immunization, Ag-specific T cells increased in number,
and a large proportion of these could be seen entering the B cell area
(Fig. 4A). In contrast, few Ag-specific T cells were observed in the
presence of abatacept, and strikingly, the majority of these were
restricted to the paracortical T cell zone. The total number of Ag-

FIGURE 2. Abatacept reduces phenotypic and functional aspects of Ag-specific T cell activation. LNs from mice that had received $1 \times 10^6$ DO11.10
T cells before immunization with OVA/CFA in the footpad and treated with PBS, control IgG, or abatacept i.p. immediately after immunization were
harvested 3 d later. Unimmunized animals received PBS. Tg T cells (CD4$^+$KJ1.26$^+$) were assessed for the expression of CD62L (A), OX40 (B), ICOS (C),
PD-1 (D), and CD28 (E). Some LN cells were also cultured with or without OVA peptide (323–339; 1 μg/ml) for 48 h in vitro, and supernatants were
analyzed by Luminex. The mean ± SD production of IFN-γ (F), IL-17 (G), IL-13 (H), and MIP-1α (I) is shown. Data are representative of at least two
independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001; Student two-tailed unpaired t test. ns, not significant.
FIGURE 3. Abatacept fails to alter T cell velocity or meandering in dLNs. Mice received $5 \times 10^6$ CMTPX-labeled DO11.10 SCID T cells before immunization with OVA/CFA in the footpad and were treated with control IgG or abatacept i.p. immediately after immunization. Popliteal LN were removed 24 h later and imaged ex vivo, as described in Materials and Methods. Images shown are z-projections of 28 sequential image stacks 1.95 µm apart and are representative of those seen in at least three fields of view in at least six animals per group. Movement of T cells is shown in Supplemental Videos 1 and 2. Mean velocity (A) and meandering indices (B) were analyzed using Volocity software programs. C–E. The mean proportion of cells in clusters (C), the number of cells in cell clusters (D), and the mean number of cells per cluster (E) were calculated as described in Materials and Methods. Data are representative of at least four independent experiments. ns, not significant; Student two-tailed unpaired t test.

specific T cells entering B cell follicles was clearly reduced after abatacept treatment, in part because of its dramatic effects on proliferation; however, the proportion of T cells in follicles was also reduced. The localization of Tg T cells was quantified using Volocity software by calculating the proportion of T cells in the follicle using T cell numbers in the follicle versus the total number of T cells. Replicate samples showed that a significantly smaller proportion of Ag-specific T cells entered B cell follicles in the presence of abatacept, decreasing from 27.00 ± 5.78% with control IgG to 14.62 ± 3.87% with abatacept treatment (Fig. 4B). Prompted by the observation of this failure in follicular migration, we assessed whether abatacept reduced the presence of Ag-specific T cells that had acquired a phenotype associated with follicular migration. Abatacept totally abrogated the presence of Ag-specific T cells that expressed CXCR5 and ICOS, markers associated with follicular migration (26) (Fig. 4C).

Abatacept reduced Ab production independently of B cell clonal expansion

We demonstrated previously in infectious diseases, such as malaria, that reduced migration of T cells into B cell follicles results in suppressed B cell responses to heterologous Ags (27). Moreover, CTLA-4Ig was reported to suppress Ab production following immunization with sheep erythrocytes and keyhole limpet hemocyanin (19). However, the mechanism by which CTLA-4Ig exerts these effects, and its potential effects on other aspects of B cell responses, remains unclear. Therefore, we assessed the impact of the impaired ability of Tg T cells to migrate into B cell follicles in the presence of abatacept on Ag-specific B cell responses. We approached this using a Tg system that allows measurement of systemic anti-HEL B cell responses, which are dependent upon help from Tg T cells (25). We cotransferred OVA-specific Tg T cells and HEL-specific Tg B cells into naive IgHb recipients and then immunized them with an OVA-HEL conjugate prepared in CFA. Following immunization, anti-HEL IgM* serum titers were significantly reduced by a single treatment with abatacept at the time of immunization (Fig. 5A). The endogenous anti-HEL response was negligible by day 5 postimmunization, because serum anti-HEL IgM* was undetectable (data not shown). However, the endogenous B cell response to OVA was detectable by day 5 postimmunization; although anti-OVA IgG2a responses were undetectable, increased levels of anti-OVA IgG1 responses were observed in immunized animals (Fig. 5B, data not shown). Similarly, the endogenous anti-OVA IgG1 response was significantly reduced after abatacept treatment (Fig. 5B). Interestingly, although abatacept reduced the number of CD4*KJ1.26* Tg T cells in the dLN as expected, it did not significantly alter the clonal expansion of IgMa* B cells 5 d after immunization (Fig. 5C, 5D). Because the effect of abatacept on Ab production seemed to be independent of clonal expansion, we next assessed whether the formation of germinal centers (GCs) was affected. Somewhat surprisingly, the percentage of Ag-specific B cells that expressed the GC B cell markers GL7 and Fas was not significantly reduced by abatacept, as assessed by flow cytometry (Fig. 5E). Together, these results suggest that abatacept modulates the function of B cells rather than reduces B cell numbers.

Abatacept prevented breach of B cell self-tolerance in arthritis

In light of the effects of abatacept on T cells, TFH cells, and B cells, as well as its therapeutic application, we next assessed its impact on these parameters in a novel model of RA. In this model, breach of self-tolerance is characterized by the spontaneous development of an autoimmune response against articular Ags, such as type II collagen (CII) (21). Following challenge with HAO, abatacept treatment reduced footpad swelling and clinical scores compared with control IgG (Supplemental Fig. 1). Popliteal LNs were
harvested, and T cell recall responses to OVA were assessed. In vitro recall anti-OVA responses by CD4+ T cells were greater in mice that had been challenged with HAO compared with PBS, indicating increased expansion and/or recruitment following secondary Ag exposure (Fig. 6A). Animals treated with abatacept manifested significantly reduced T cell proliferative responses to OVA (Fig. 6A). In addition, the proportion of total CD4+ T cells expressing CXCR5 was significantly reduced by treatment with abatacept in vivo (Fig. 6B).

We measured autoreactive Ab levels to assess the impact of abatacept on breach of B cell self-tolerance. Following HAO challenge, anti-CII IgG responses increased compared with PBS challenge, indicating a breach of tolerance toward CII (Fig. 6C), as reported previously (21). Strikingly, and in contrast to HAO-challenged, control IgG-treated animals, treatment with abatacept prevented the breach of B cell tolerance, with levels of anti-CII IgG comparable to PBS-challenged animals. Interestingly, recall Ab responses to OVA were also significantly reduced by treatment with abatacept (Fig. 6D). Moreover, abatacept treatment significantly reduced the proportion of GL7+Fas+ GC B cells in the LN draining the affected joint after HAO challenge (Fig. 6E, 6F).

Discussion
Abatacept has proven efficacious in the treatment of RA, but its mode of action in vivo remains to be fully elucidated. Indeed, its effects on specific T cell responses and the development of specific T-dependent B cell responses in vivo are unclear, as are its effects on memory cell responses and breach of tolerance to self, joint-associated Ags. In this article, we provide evidence that, in addition to inhibiting T cell activation and proliferation, abatacept reduces the migration of T cells into B cell follicles. Importantly, this results in reduced Ab responses, independently of B cell clonal expansion. This failure of T cell help for B cells and the subsequent Ab response are recapitulated in a novel model of RA in which abatacept prevents breach of self-tolerance, as well as reduces anti-self Ab responses and associated pathology.

Abatacept was demonstrated to inhibit T cell proliferation in in vitro human MLRs and in alloresponses in vivo (12, 18). In this study, we examined its effects on Ag-specific T cell responses in vivo; abatacept dramatically reduced proliferation after challenge with Ag in adjuvant. Furthermore, abatacept reduced the downregulation of CD62L expression, a characteristic of activated T cells that is needed for cells to exit the LN to migrate to tissue sites of inflammation (28). The modulation of CD62L expression suggests abatacept could inhibit the migration of T cells into inflamed tissues. As expected (29), Ag-specific T cells increased their expression of OX40, which is downstream of CD28, after challenge with Ag in adjuvant. However, treatment with abatacept suppressed the expression of OX40, which could culminate in reduced survival of specific T cells as a result of the reduced expression of the antiapoptotic factors Bcl-2 and Bcl-xL (30). Interestingly, OX40 also controls the expression of survivin, which maintains T cell division over time (31). These findings are consistent with a previous report demonstrating reduced CD28-
of cells per cluster, but this was not significant. However, as we
ever, when we analyzed T cell clustering, there was a trend toward
meandering following immunization with Ag in adjuvant. How-
cept did not significantly alter Ag-specific T cell velocity or
mation of DC–T cell clusters of smaller sizes and interactions of
human tonsils (44), and this was significantly reduced by treat-
Interestingly, IL-13 was shown to be produced by TFH cells in
inhibition of Ag-specific T cell IL-17 production by abatacept.
several inflammatory diseases, including RA (40–42). Although
Abatacept also significantly reduced the upregulation of PD-1, a
negative regulator of T cell activation (37), by activated Ag-
specific T cells in situ, we observed that in abatacept-treated animals,
DCs and T cells will allow more detailed analyses of the effects of
abatacept on cellular interactions.

However, when we examined the localization of Ag-specific
T cells in situ, we observed that in abatacept-treated animals,
the majority of cells were restricted to the para cortical T cell zone
even following immunization. The total number of Ag-specific
T cells entering B cell follicles was clearly reduced after abatacept
treatment, in part because of its dramatic effects on proliferation;
however, the proportion of T cells in follicles was also reduced.
Interestingly, this lack of TFH cells could also be related to the in-
hibition of proliferation, because far fewer cells had undergone four
or more divisions, which is a characteristic of this more-differentiated
subset (46). The lack of follicular migration was also associated with suppression of the acquisition of a CXCR5+
ICOS+ TFH phenotype among the Tg population. Failed migration
likely reflects the inability of Ag-specific T cells to migrate to
CXCL13 via CXCR5.

We demonstrated previously in infectious diseases, such as
malaria, that reduced migration of T cells into B cell follicles
results in suppressed B cell responses to heterologous Ags (27).
Therefore, we assessed the impact of the impaired ability of Tg
T cells to migrate into B cell follicles in the presence of abatacept
on Ag-specific B cell responses. CTLA-4Ig was reported to sup-
press Ab production following immunization with sheep erythro-
cytes and keyhole limpet hemocyanin (19). Although abatacept
suppressed the proliferation of Tg T cells in these experiments,
we also demonstrated that it reduced the systemic Ab response
without affecting the clonal expansion of Tg B cells. Together,
these results suggest that abatacept acts to modulate the function
of B cells rather than reduce B cell numbers. It remains possible
that in the T and B cell cotransfer experiments, T cell migration
into follicles was increased as a result of the effects of chemotactic
factors, such as CXCL13, which can be expressed by activated

FIGURE 5. Abatacept reduces Ab production independently of B cell clonal expansion. A, B, Naive recipients were adoptively transferred with 1 × 10^6
DO11.10 T cells and 1 × 10^6 MD4 B cells 1 d prior to immunization with OVA-HEL/CFA. Mice were treated with PBS, control IgG, or abatacept i.p.
immediately after immunization. Serum was taken from animals 5 d after immunization and assessed for the presence of anti-HEL IgMa (A) and anti-OVA
IgGl (B) by ELISA. C, D, LNs were also taken 5 d later and stained for the presence of Tg T cells and Tg B cells by flow cytometry. Results shown are the
mean number ± SD of CD4+KJ1.26+ cells (C) and B220+IgMa+ cells (D) for three mice/group. E, The mean proportion ± SD of GL7+ Fas+ Tg B cells
(B220+IgMa+) 5 d postimmunization, as assessed by flow cytometry. Results are representative of two or three independent experiments. *p < 0.05; **p <
0.01; ***p < 0.001; Bonferroni-corrected two-way ANOVA (A, B) or Student two-tailed unpaired t test (C, D). ns, not significant.
B cells (47). It is also interesting to speculate that because B cells express CD80/CD86, abatacept could prevent phenotypic maturation of B cells, which may relate to the reduced levels of Ab production observed. We then investigated whether this failure of T cell help for B cells and the subsequent Ab response were recapitulated in a novel OVA-driven model of RA. We previously described the suggested mode of action of this model in detail (21, 48–50). Briefly, we think that by transferring a large number of activated Th1 cells of an irrelevant specificity and inducing a local inflammatory stimulus in the joint, the normal regulatory mechanisms are overwhelmed and breach of self-tolerance occurs. We suggested previously that the inflammation initiated by OVA-specific T cells causes normally quiescent, tolerogenic DCs carrying self-Ag (collagen) from the joint to the local LN to present this in an immunogenic manner to self-reactive T cells, thereby breaking tolerance and initiating autoimmunity. In this study, we demonstrated that abatacept reduced the clinical scores following challenge. Abatacept also prevented breach of B cell tolerance, inhibiting the generation of anti-CII IgG Abs. Strikingly, abatacept also reduced anti-OVA IgG2a recall responses. In addition,

FIGURE 6. Abatacept prevents the breach of self-tolerance in experimental arthritis. A, plNs from unchallenged, challenged/PBS, challenged/control IgG, and challenged/abatacept mice were harvested 7 d after footpad challenge. Mice received PBS/control IgG/abatacept i.p. on days −2, 0, 2, 4, and 6, with HAO injection on day 0. HAO was given alone by footpad injection. Total LN cells were restimulated in vitro with medium or OVA. Results shown are the mean percentage ± SD of total CD4+ cells that had incorporated EdU (5-ethyl-2'-deoxyuridine) for five mice per group. B, Percentage of total CD4+ cells that was CXCR5+ in each condition. Serum samples were assayed for the presence of total anti-CII IgG (C) and anti-OVA IgG2a (D) by ELISA. E and F. The percentage of B220+ cells that was Fas+GL7+ was assessed; results are shown as representative staining of B220 gated cells (E) and the mean percentage ± SD of B220+ cells that were Fas+GL7+ for five mice per group (F). Results are representative of two independent experiments. **p < 0.01; ***p < 0.001; Bonferroni-corrected two-way ANOVA (C, D) or a Student two-tailed unpaired t test (A, B, F). ns, not significant.
abactept dramatically reduced the number of GC B cells in the LN draining the affected joint, providing a reason for the diminished anti-OVA and anti-ClI Ab levels.

Abactept has proven efficacious in the treatment of RA, but its mode of action in vivo remains to be fully elucidated. Its effects on specific T cell responses and the development of specific T-dependent B cell responses in vivo remained unclear, as were its effects on memory cell responses and the breach of tolerance to self, joint-associated Ags. We provide novel data that directly inform these questions. Important effects of abactept on the development of TFH cells and the development of B cell responses were observed. Abactept prevents full T cell proliferation and activation; importantly, it also blocks follicular migration of T cells.

In turn, cognate B cells, although able to undergo normal clonal expansion, have a reduced capacity to produce Abs. The inhibition of B cell responses also occurs during experimental arthritis, in which the endogenous breach of B cell self-tolerance is prevented, as reflected by reduced GC formation and reduced levels of autoantibodies. Together, these experiments provide, for the first time, a mechanistic insight into how abactept suppresses T cell responses, as well as T-dependent B cell responses, in vivo. This, in turn, provides strong rationale for early application of abactept in the disease course of RA.

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Disclosures
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References


