Syk Tyrosine Kinase Is Critical for B Cell Antibody Responses and Memory B Cell Survival

Jochen A. Ackermann, Josquin Nys, Edina Schweighoffer, Scott McCleary, Nicholas Smithers and Victor L. J. Tybulewicz

*J Immunol* 2015; 194:4650-4656; Prepublished online 10 April 2015;
doi: 10.4049/jimmunol.1500461
http://www.jimmunol.org/content/194/10/4650

**Supplementary Material**
http://www.jimmunol.org/content/suppl/2015/04/10/jimmunol.1500461.DCSupplemental

**References**
This article cites 22 articles, 7 of which you can access for free at:
http://www.jimmunol.org/content/194/10/4650.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Syk Tyrosine Kinase Is Critical for B Cell Antibody Responses and Memory B Cell Survival

Jochen A. Ackermann,* Josquin Nys,* Edina Schweighoffer,* Scott McCleary,* Nicholas Smithers,† and Victor L. J. Tybulewicz*

Signals from the BCR are required for Ag-specific B cell recruitment into the immune response. Binding of Ag to the BCR induces phosphorylation of immune receptor tyrosine-based activation motifs in the cytoplasmic domains of the CD79a and CD79b signaling subunits, which subsequently bind and activate the Syk protein tyrosine kinase. Earlier work with the DT40 chicken B cell leukemia cell line showed that Syk was required to transduce BCR signals to proximal activation events, suggesting that Syk also plays an important role in the activation and differentiation of primary B cells during an immune response. In this study, we show that Syk-deficient primary mouse B cells have a severe defect in BCR-induced activation, proliferation, and survival. Furthermore, we demonstrate that Syk is required for both T-dependent and T-independent Ab responses, and that this requirement is B cell intrinsic. In the absence of Syk, Ag fails to induce differentiation of naïve B cells into germinal center B cells and plasma cells. Finally, we show that the survival of existing memory B cells is dependent on Syk. These experiments demonstrate that Syk plays a critical role in multiple aspects of B cell Ab responses. The Journal of Immunology, 2015, 194: 4650–4656.
more, by deleting Syk after a primary immunization, we show that the kinase is also required for the secondary memory Ab response. Finally, we show that Syk is required for the survival of memory B cells.

Materials and Methods

Mice carrying a conditional allele of Syk (Sykfl/fl), a constitutive disruption of Syk (Sykldtm1Lnes Syk1), an allele of ROSA26 expressing the MerCreMer fusion protein (Gt(RosA26)2Sortm1cre/+;Nes; Rosa26MerCreMer; RMCM), disruption of the IgM gene (Igmlm1Cpsrm1Cpsm), and a disruption of Ragl (Rag1tm1Shkm) have been described previously (10, 13, 14). SWHEL mice, expressing an anti-hen egg lysozyme (HEL) BCR were a gift from R. Brink and were backcrossed into the Igh alleles (Ighm, Igh1, Igh2, and an Ig L chain transgene (TygIghHEL10Ihrb) bred together to generate the SWHEL strain (Ighm1Shkm1IhrbIgh10IhrbIgh10Ihrb). (15). All strains were bred on a C57BL/6N background. B6.SJL and (B6 x B6.SJL)F1 mice were obtained from the breeding facility at the National Institute for Medical Research. To induce Cre activity, mice were injected i.p. for 5 d with 2 mg/day of tamoxifen (Sigma).

To generate mixed radiation chimeras, bone marrow was harvested from Sykfl/fl-RMCM mice or Sykfl/fl-RMCM mice, treated with ACK lysis buffer and mixed in a 1:2 ratio with bone marrow cells from μMT (Igmlm1Cpsrm1Cpsm) mice, and injected intravenously at 1 × 106 cells per recipient into Ragl-deficient animals that were irradiated with 5 Gy using a 137Cs source. Mice received Baytril in their drinking water (0.02%, Bayer Healthcare) for at least 4 wk after transplantation, and were used for further studies no less than 6 wk after reconstitution.

Cell culture

Splenic B cells were purified from mice treated 21 d earlier with tamoxifen, by magnetic negative depletion using biotinylated Abs against CD3ε (eBioR2/60), CD11c (N418), CD11b (M1/70), and CD3ε (145-2C11; eBioscience) and streptavidin Dynabeads (Life Technologies). B cells (1 × 106 cells/ml) were incubated in culture containing 0.5 μM CFSE (Molecular Probes) at 37˚C for 10 min. Cells were washed twice in DMEM plus medium (DMEM with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μM nonessential amino acids, 20 mM HEPES buffer, 2 mM l-glutamine, and 50 mM 2-ME) and then cultured at 1–1.5 × 106 cells/ml in DMEM-plus alone or supplemented with 10 μg/ml anti-IgM Fab2 (Jackson ImmunoResearch) or 100 ng/ml CD40L (R&D Systems) and 20–100 ng/ml IL-4 (PeproTech). Flow cytometry was used to assess surface expression of proteins, proliferation by CFSE dilution, and number of live cells by a LIVE/DEAD fixable near-infrared dead cell stain.

Immunizations

For T-independent responses, mice were immunized i.p. with 10 μg TNP-Ficoll. For T-dependent immune responses, mice were immunized i.p. with 50–100 μg Alum-precipitated (Thermo Scientific) 4-hydroxy-3-nitrophenylacetyl conjugated to chicken-y-globulin (NP-CCG), with a ratio of NP to CGG ranging from 21 to 27 (BioSearch Technologies). For recall, responses mice were injected i.p. with 50 μg NP-CCG in PBS. TNP or NP-specific Abs in the serum were measured by ELISA using Maxisorp plates (Nunc) coated with NPε-BSA as previously described (16).

Flow cytometry

Single-cell suspensions of splenocytes were treated with ACK lysis buffer to remove RBCs before staining in PBS, containing LIVE/DEAD fixable near-IR dead cell stain (Life Technologies) and appropriate, pretreated Abs. Abs used, indicating Ag and fluorophore (and clone): CD69-FITC (H1.2F3), CD86-PE (GL-1), streptavidin-PerCP (Becton Dickinson), MHC class II-bio (I-Ab; Serotec). Ab staining of intracellular Syk was performed as described previously (18). The germinal center reaction was blocked by three injections of 300 μg anti-CD40L (MR1) or control Hamster IgG (BioXcell) every second day.

Statistical analysis

All statistical comparisons used the nonparametric two-tailed Mann–Whitney U test or Student t test. Statistically significant differences are indicated in the figures.

Results

Syk is required for in vitro BCR-induced activation

Initially, we investigated whether Syk was required for Ag receptor-induced activation of B cells in vitro, using mice containing a conditional allele of Syk in which exon 11 is flanked by loxP sites (Sykfl) and a tamoxifen-inducible Cre recombinase expressed from the ROSA26 locus (Rosa26MerCreMer; RMCM) (13). These strains and mice bearing a deleted allele of Syk (Syk−/−) (10) were intercrossed to generate control (Sykfl/+RMCM) and conditional mutant (Syk−/−RMCM) mice. Treatment with tamoxifen resulted in deletion of the Sykfl allele in both strains, leaving control and mutant mice with either one or no functional alleles of Syk. As established previously, by 10 d after tamoxifen treatment, Syk protein is no longer detectable in splenic B cells from mutant mice, and by 21 d ~80% of the B cells are lost because of impaired survival (13). Despite this loss of B cells, ~20% of follicular B cells persist in the mutant but have no detectable Syk. Thus, we were able to use these Syk-deficient B cells to investigate whether Syk was required for BCR-induced activation in vitro. We found that Syk was required for BCR-induced increase in cell surface expression of CD69, CD86, and MHC class II proliferation and survival (Fig. 1A, 1B). In contrast, Syk-deficient B cells were able to respond at least as well as control cells to stimulation with CD40L plus IL-4, as measured by the same parameters, demonstrating that Syk-deficient cells retained the capacity to become activated and to proliferate. However, Ig isotype class switching to IgG1 and secretion of IgM and IgG1 in response to CD40L plus IL-4 were greatly reduced in the absence of Syk (Fig. 1C, 1D). Thus, Syk was required in all BCR-induced in vitro activation responses we measured, but only in a select subset of responses induced by CD40L plus IL-4.

Defective T-independent responses in the absence of Syk

Next, we investigated the requirement for Syk during in vivo B cell responses to Ag. The Rosa26MerCreMer allele is expressed ubiquitously; therefore, to rule out secondary effects on Ab responses because of deletion of Syk in non–B cells, we limited the loss of Syk to B cells by generating mixed chimeras in which the hematopoietic system of irradiated Rag1-deficient mice was reconstituted with

with DMEM. Splenocytes or bone marrow cells were serially diluted (maximum concentration of 4 × 105 cells/well) on the coated plates and cultivated overnight in DMEM-plus with 5% FCS at 37˚C. 5% CO2. Plates were washed twice with PBS, 0.05% Tween-20 and twice with PBS, stained with biotinylated anti-IgG1 or anti-IgM Abs for 2 h at room temperature, washed four times with PBS-Tween, and stained with Avidin–alkaline phosphatase for 1 h at room temperature. Alkaline phosphatase activity was visualized using BCIP/NPT substrate (BioFX). Spots, each representing a single Ab-secreting cell, were counted using an ImmunoSpot reader.

Cell transfers and immunization with HEL

To induce primary responses 2.5 × 108 HEL-binding B cells from tamoxifen-treated SWHEL/Syklfl or SWHEL/Syk−/−RMCM mice were transferred into B6.SJL recipient mice together with 5 × 108 HEL-conjugated sheep RBCs (SRBC). To generate a memory pool, splenocytes containing an equivalent of 5 × 107 HEL-binding B cells from SWHEL/Sykfl/RMCM or SWHEL/Syk−/−RMCM mice were transferred into (B6 x B6.SJL)F1 recipient mice together with 1 × 109 HEL3−conjugated SRBC. HEL (Sigma–Aldrich) or HEL3− (a gift from R. Brink) (17) was conjugated to SRBC (Patricell) as described previously (18). The germinal center reaction was blocked by three injections of 300 μg anti-CD40L (MR1) or control Hamster IgG (BioXcell) every second day.

Statistical analysis

All statistical comparisons used the nonparametric two-tailed Mann–Whitney U test or Student t test. Statistically significant differences are indicated in the figures.
a mixture of bone marrow from μMT (B cell–deficient) mice and either Syk-expressing (Syk<sup>fl/+</sup>RMCM) or Syk-deficient (Syk<sup>fl/2</sup>RMCM) mice. In the resulting mutant mice, all B cells lost Syk expression after tamoxifen treatment, whereas Syk was maintained in other cell types. Initially, we determined whether Syk was required for T-independent type-II (TI-II) responses to TNP-Ficoll, a hapten coupled to highly repetitive polysaccharide, by immunizing chimeric mice 21 d after treatment with tamoxifen. We found that in the absence of Syk, there was a significant reduction in Ag-specific IgM and IgG3 (Fig. 2), demonstrating a B cell-intrinsic requirement for Syk in TI-II Ab responses.

Defective T-dependent Ab response in the absence of Syk in B cells

To determine whether Syk was also required for T-dependent B cell responses, Syk<sup>fl/+</sup>RMCM and Syk<sup>fl/2</sup>RMCM mice were treated with tamoxifen after 21 d with NP-CGG precipitated in alum, and then analyzed after an additional 10 d (Fig. 3A). We found that loss of Syk resulted in a significant decrease in Ag-specific IgM, IgG1, IgG2b, and IgG2c in the serum, and in the induction of far fewer germinal center B cells (Fig. 3B). Notably, although most B cells in the mutant mice had no detectable Syk expression, the few remaining germinal center B cells in the same mice still expressed Syk (Fig. 3B). This strong selection against cells that have deleted Syk indicates that B cells require Syk to differentiate into germinal center cells.

To evaluate whether the requirement for Syk in T-dependent B cell responses was B cell-intrinsic, we immunized chimeric mice with NP-CGG. We found that restricted loss of Syk in B cells resulted in a large decrease in Ag-specific IgM and IgG1 in the serum (Fig. 3C), and a large reduction in the numbers of Ag-specific IgG1-expressing B cells and germinal center B cells, as well as a reduction in Ab-secreting cells producing Ag-specific IgM or IgG1 (Fig. 3D).

FIGURE 1. Syk is required for activation of B cells in response to anti-IgM and class switching in response to CD40L + IL-4. (A) Mean (±SEM) expression of CD69, CD86, and MHC class II on B cells of the indicated genotypes stimulated with anti-IgM or CD40L plus 100 ng/ml IL-4 or left unstimulated (−) for 36 h. (B) Mean (±SEM) percentage of divided cells and percentage of live cells in cultures of B cells stimulated as in (A) for 60 h. (C) Dot plots of surface staining of IgM and IgG1 on B cells of the indicated genotypes stimulated for 60 h with CD40L plus 20 ng/ml IL-4 or left unstimulated (−). Numbers indicate the percentage of cells falling into the gate. Graph of mean (±SEM) percentage of IgG1<sup>+</sup> B cells in cultures, determined as in dot plots. (D) Mean (±SEM) levels of IgM and IgG1 secreted into the medium of B cells cultured as in (C) for 6 d. *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant.

FIGURE 2. Defective T-independent Ab response in the absence of Syk in B cells. Irradiated Rag1-deficient mice reconstituted with a mixture of bone marrow from μMT (B cell–deficient) bone marrow and Syk-expressing (Syk<sup>fl/+</sup>RMCM) or Syk-deficient (Syk<sup>fl/2</sup>RMCM) bone marrow were treated with tamoxifen, immunized 21 d later with TNP-Ficoll, and analyzed 10 d later. Graphs show levels of anti-TNP IgM or IgG3 in the serum of the mice (dots, individual mice; line, mean). **p < 0.01. AU, arbitrary units.
FIGURE 3. Defective T-dependent Ab response in the absence of Syk in B cells. (A and B) Syk-expressing (Syk$^{fl/+}$RMCM) or Syk-deficient (Syk$^{fl/-}$RMCM) mice were treated with tamoxifen, immunized 21 d later with NP-CGG in Alum, and analyzed 10 d later. (A) Graph shows anti-NP Ig of the indicated isotype in serum of mice of the indicated genotypes either just before immunization (day 0) or 10 d later (dots, individual mice; line, mean). (B) Dot plots of splenocytes from mice 10 d after immunization gated on B220+ cells showing staining with Abs to Fas and GL7. Numbers indicate the percentage of cells falling into each gate. Column graph shows levels of Syk in T cells (B220$^{-}$), B cells (B220$^{+}$) and germinal center B cells (GCs). T cells show no detectable Syk by this assay. (C and D) Irradiated Rag1-deficient mice reconstituted with a mixture of $\mu$MT bone marrow and Syk-expressing (Syk$^{fl/+}$RMCM) or Syk-deficient (Syk$^{fl/-}$RMCM) bone marrow were treated with tamoxifen, immunized 21 d later with NP-CGG in alum or alum alone, and analyzed 10 d after that. (C) Graph of anti-NP IgM or IgG1 in mice of the indicated genotypes (dots, individual mice; line, mean). (D) Mean ($\pm$SEM) number of Ag-specific switched B cells (B220$^{+}$NP-IgG1+) or germinal center B cells (B220$^{+}$Fas$^{+}$GL7$^{+}$NP-IgG1$^{+}$; Supplemental Fig. 1A) or Ab-secreting cells (ASCs) making NP-specific IgM or IgG1. (E) SWHEL Syk$^{fl/}$ or SWHEL Syk$^{fl/-}$RMCM mice were treated with tamoxifen and 21 d later B cells were transferred into B6.SJL mice. Recipient mice were immunized with SRBC or HEL-SRBC and analyzed 5 d later. Graphs show mean ($\pm$SEM) number of total splenic donor-derived B cells, non-GC B cells, GC B cells, and plasma cells (Supplemental Fig. 1B). *p < 0.05, **p < 0.01, ***p < 0.001.
The reduced T-dependent B cell response could have been caused by a requirement for Syk in B cells to differentiate into germinal center and plasma cells. Alternatively, it could have resulted from Syk deficiency reducing the number of naive B cells, leading to a lower number of precursor cells able to respond to the immunizing Ag. To address this issue, we bred the conditional Syk mutation to SWHEL mice expressing a BCR-specific for HEL (15). In this strain, a recombinated VDJ region from a HEL-specific Ab has been inserted into the IgH locus, and the mice also contain a transgene expressing an Igκ L chain from the same Ab. We transferred equal numbers of control and Syk-deficient SWHEL B cells into wild-type recipients, and immunized them with HEL-coupled SRBC or SRBC alone (Fig. 3E). Analysis 5 d later showed that control B cells expanded strongly in mice that were immunized with HEL-SRBC compared with SRBC alone, but that this did not occur in the absence of Syk (Fig. 3E). Subdividing these B cells into nongerminal center and germinal center B cells showed the same result—a large expansion of control Syk-expressing B cells, but not of mutant Syk-deficient cells. Ag-induced expansion of plasma cells was also strictly Syk-dependent. Therefore, B cell–expressed Syk is required for the Ag-induced expansion of B cells and their differentiation into germinal center and plasma cells in response to a T-dependent Ag.

Defective Ab recall response in the absence of Syk in B cells

A characteristic feature of the adaptive immune response to T-dependent Ags is a secondary, or recall, response that is typically faster and stronger than the primary response. This is due to the formation of memory B and T cells during the primary response, which respond more rapidly and more strongly to Ag than naive lymphocytes (19). To evaluate whether Syk is also required

![Figure 4](https://example.com/figure4.png)

**FIGURE 4.** Defective Ab recall response in the absence of Syk in B cells. (A and B) Irradiated Rag1-deficient mice reconstituted with a mixture of μMT bone marrow and Syk-expressing (Syk\(^{fl/+}\)RMCM) or Syk-deficient (Syk\(^{fl/-}\)RMCM) bone marrow were immunized twice with NP-CGG in alum. Eighty-five days after the second immunization, the mice were treated with tamoxifen; 21 d later they were immunized with NP-CGG in PBS or PBS alone and analyzed 5 d after that. Graphs show mean (±SEM) numbers of Ag-specific switched B cells (NIP+IgD\(^{-}\); a mix of memory, germinal center, and plasma cells) and plasma cells in the spleen (Supplemental Fig. 2). Histograms show levels of Syk in T cells, all B cells, and Ag-specific switched B cells or plasma cells (PCs). (B) Mean (±SEM) numbers of splenic Ab-secreting cells (ASCs) making NP-specific IgM or IgG1. (C) Mice were immunized twice with NP-CGG in alum as in (A); 39 d later they were treated with tamoxifen and 21 d later were challenged with NP-CGG in PBS or PBS alone. Dot plots show binding of Ag (NIP) and expression of surface IgG1 on splenic B cells (B220\(^{+}\)). Numbers indicate the percentage of cells falling into gate. The graph shows mean (±SEM) numbers of Ag-specific IgG1-expressing B cells (B220\(^{+}\)NIP+IgG1\(^{+}\)). \(*p < 0.05, **p < 0.01.\)
by memory B cells for their response to Ag, we immunized chimeric mice in which deletion of Syk could be limited to the B cell lineage, but that had not yet been treated with tamoxifen. The mice were immunized twice with NP-CGG precipitated in Alum, treated with tamoxifen 53 or 99 d later to delete Syk in B cells, and given a secondary challenge of NP-CGG in PBS or just PBS alone a further 21 d later (Fig. 4A). Analysis of splenocytes 5 d after this secondary boost showed that although the number of control Ag-specific switched B cells or plasma cells increased greatly in response to the secondary challenge with NP-CGG, in the absence of Syk this expansion was largely absent (Fig. 4A). Notably, the few switched B cells or plasma cells seen in these mice still expressed Syk, showing again a strong selection against the loss of Syk in responding B cells. Further analysis showed similar large reductions in Ab-secreting cells making NP-specific IgM and IgG1 and in Ag-specific switched IgG1+ B cells (Fig. 4B, 4C). These results indicate that the absence of Syk in B cells greatly diminishes the secondary recall response.

Syk is required for the survival of memory B cells

This requirement for Syk in the secondary T-dependent response could have been due to a role for Syk within memory B cells for their proliferation and differentiation into plasma cells. Alternatively, Syk could have been required for the survival of memory B cells. We have previously shown that the survival of naive B cells is dependent on Syk, in part because it transduces survival signals from BAFFR and the BCR (13). However the signals controlling the survival of memory B cells are largely unknown. It has been established that BAFF is not required for their survival (20), but it is not known whether the BCR is required. Thus, we next investigated the potential role for Syk in memory B cell survival. We again made use of SWHEL mice bred to either control or conditional Syk mutant mice and transferred equal numbers of SWHEL B cells from each strain into wild-type recipients, and immunized with HEL3×-SRBC or SRBC alone (Fig. 5). HEL3× is a variant of HEL with a lower affinity for the anti-HEL BCR in SWHEL mice that gives longer-lasting immune responses (17). The donor mice were not treated with tamoxifen; therefore, the SWHEL B cells from both control and conditional mutant mice still expressed Syk and thus could mount a strong immune response, including formation of similar numbers of memory B cells (not shown). Fifty-four days after immunization, the mice were treated with tamoxifen to delete Syk in the conditional mutant memory B cells, and the numbers of these cells was evaluated 21 d

**FIGURE 5.** Syk is required for the survival of memory B cells. B cells from SWHEL Syk+/+RMCM or Sykfl/flRMCM mice were transferred into (B6 x B6.SJL) F1 mice, and recipient mice were immunized with SRBC or HEL3×-SRBC. After 48 d, mice were given three injections of anti-CD40L (MR1) or an isotype control Ab and then tamoxifen 54 d after immunization, and they were analyzed 21 d later. Dot plots in the top row show expression of CD38 and Fas on donor-derived splenic B cells (CD45.1+B220+), and the gate indicates naive and memory B cells (CD38+Fas−); these in turn were analyzed for expression of IgD and IgG1 in the lower dot plots, where the gate shows donor-derived IgG1+ memory B cells. Numbers indicate the percentage of cells falling into the gate. The graph shows the number of splenic donor-derived IgG1+ memory B cells, with each dot representing a single mouse and the line showing the mean. **p < 0.01.
later. Importantly, just before treatment with tamoxifen, the mice were given three injections of an anti-CD40L Ab to block the germinal center reaction. Because some germinal center B cells differentiate into memory B cells, and Syk is required for the formation of germinal center B cells, we needed to ensure that any potential drop in memory B cell numbers following deletion of Syk was not due to the loss of germinal center cells. The anti-CD40L treatment effectively eliminated all germinal center cells (compared with treatment with isotype control Ab; Fig. 5). Analysis of memory B cell numbers in mice immunized with HEL3xSRBC showed that deletion of Syk resulted in a large reduction in the number of IgG1+ memory B cells (Fig. 5). Thus, Syk is required for the survival of memory B cells.

Discussion

Our results establish that Syk is a key signal transducer that is required for coupling the BCR to all downstream signaling pathways in mature B cells. All measures of activation including up-regulation of cell surface markers, proliferation, and survival were dependent on Syk expression. In contrast, Syk-deficient B cells could be efficiently activated by CD40L plus IL-4, as measured by the same parameters, although class switching was defective, suggesting that Syk must contribute in some way to this process. We have shown previously that survival signals from BAFFR are partially transduced via the BCR to the activation of Syk (13). By analogy, signals from CD40 or IL-4R may be transduced in part via the BCR and Syk. We note that both CD40 and BAFFR are members of the TNFR family and signal by similar mechanisms.

Our work also demonstrates that Syk is absolutely required for both TI-II and T-dependent Ab responses. In particular, Syk-deficient B cells cannot differentiate into germinal center or plasma cells, suggesting that BCR-derived signals transduced via Syk are essential for these differentiation events. A recent study showed that LPS upregulation of the Blimp1 transcription factor in B cells in vitro requires Syk expression (21). Blimp1 is required for full differentiation into plasma cells (22). Our results therefore raise the possibility that the LPS receptor TLR4 also transduces signals via the BCR and Syk to induce Blimp1 transcription.

Finally, we showed that Syk is needed for the survival of memory B cells, a process about which little is known. This requirement for Syk suggests that an ITAM-bearing receptor is also likely to operate in memory B cells. However the cytokines BAFF and APRIL are not required for the survival of memory B cells (20), suggesting that if such a BCR/Syk survival pathway operates in memory B cells, it may be downstream of a different cytokine receptor.

Acknowledgments

We thank S. Ley for critical reading of the manuscript, R. Brink for mice, and the Biological Services Division and Procedural Services Section of National Institute for Medical Research for antibody husbandry and technical support.

Disclosures

S.M. and N.S. are employees of GlaxoSmithKline. The other authors have no financial conflicts of interest.

References