TACI Is Required for Efficient Plasma Cell Differentiation in Response to T-Independent Type 2 Antigens

George T. Mantchev, Catarina S. Cortesão, Michelle Rebrovich, Marilia Cascalho and Richard J. Bram

*J Immunol* 2007; 179:2282-2288; doi: 10.4049/jimmunol.179.4.2282

http://www.jimmunol.org/content/179/4/2282
TACI Is Required for Efficient Plasma Cell Differentiation in Response to T-Independent Type 2 Antigens

George T. Mantchev, Catarina S. Cortesão, Michelle Rebrovich, Marilia Cascalho, and Richard J. Bram

The control of systemic infection by encapsulated microorganisms requires T-independent type II (TI-2) Ab responses to bacterial polysaccharides. To understand how such responses evolve, we explored the function of transmembrane activator calcium modulator and cyclophilin ligand interactor (TACI), a member of the TNFR family, required for TI-2 Ab production. Quasimonoclonal (QM) mice produce robust TI-2 responses to 4-hydroxy-3-nitrophenylacetate (NP)-Ficoll, owing to the high precursor frequency of NP-specific B cells in the marginal zone of the spleen. QM mice that lack TACI produce decreased numbers of IgM (2-fold) and IgG (1.6-fold) NP-specific ASCs, compared with TACI-positive QM mice in response to immunization with NP-Ficoll. Our studies indicate that TACI acts at a remote time from activation because TACI is not necessary for activation and proliferation of B cells both in vitro and in vivo. Instead, TACI-deficient QM B cells remained in the cell cycle longer than TACI-proficient QM cells and had impaired plasma cell differentiation in response to NP-Ficoll. We conclude that TACI has dual B cell-autonomous functions, inhibiting prolonged B cell proliferation and stimulating plasma cell differentiation, thus resolving the longstanding paradox that TACI may have both B cell-inhibitory and -stimulatory functions. By promoting plasma cell differentiation earlier during clonal expansion, TACI may decrease the chances of autoantibody production by somatic hypermutation of Ig genes in response to T-independent Ags. The Journal of Immunology, 2007, 179: 2282–2288.

*Transplantation Biology Program and the Departments of Immunology, Pediatric and Adolescent Medicine, and Surgery, Mayo Clinic College of Medicine, Rochester, MN 55905

Received for publication October 19, 2006. Accepted for publication May 29, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by National Institutes of Health Grants CA76274, AI48602, AI53733, and HL79067.

2 G.M. and C.C. contributed equally to this work.

3 Address correspondence and reprint requests to Drs. Marilia Cascalho and Richard J. Bram, Mayo Clinic, 200 First Street S.W., Rochester, MN 55905. E-mail addresses: cascalho.marilia@mayo.edu and bram.richard@mayo.edu

4 Abbreviations used in this paper: TI-2, T-independent type 2; QM, quasimonoclonal; NP, 4-hydroxy-3-nitrophenylacetate; TACI, trans-membrane activator calcium modulator and cyclophilin ligand interactor; BlyS, B lymphocyte stimulator; APRIL, a proliferation inducing ligand; KO, knockout; ASC, Ab-secreting cell; Neu5Acα, α-2,6-linked N-acetylleucosaminic acid.

Copyright © 2007 by The American Association of Immunologists, Inc. 0022-1767/07/$2.00

www.jimmunol.org
The Journal of Immunology

2283

with defective TI-2 Ab responses, owing to decreased or absent B cells in the marginal zone of the spleen or in the serosal cavities (B1 B cells). In contrast to the apparent impact on TI-2 responses, TACI is thought to inhibit some B cell functions. Thus, TACI−/− B cells hyperproliferate in response to polyclonal activators in vitro, and loss of TACI causes autoimmunity in mice (6, 17, 18), suggesting that TACI is a B cell-inhibitory receptor. How TACI combines inhibitory and stimulatory B cell functions is not understood. Here we asked whether TACI regulates B cell differentiation after T-independent stimulation of B cells.

To determine the mechanism by which TACI controls TI-2 Ab responses, we bred TACI-KO mice with the QM mouse (3) to obtain TACI−/− QM mice. Because in QM mice the transgenic H chain was targeted into the JH locus, it can undergo isotype switching and somatic hypermutation. We studied how TACI promotes QM B cell responses to NP-Ficoll (a TI-2 Ag). We found that QM B cells lacking TACI are normally activated by Ag but proliferate longer and have impaired plasma cell differentiation. Our findings suggest that TACI acts remotely from B cell activation and provides a critical signal for cells to exit the cell cycle and to differentiate into plasma cells.

Materials and Methods

Mice and immunizations

TACI-KO and QM mice were previously described (3, 6). TACI-KO mice were bred to QM mice and are in a C57BL/6 (B6) background. Animals were bred and treated according to the policies of Mayo Foundation Institutional Animal Care and Use Committee. Mice were immunized with 30 μg i.p. of NP-Ficoll (NP41-AECM-Ficoll; Biosearch Technologies) diluted in sterile PBS.

Isolation of primary B cells

CD19+ splenocytes were isolated by negative selection with a B cell isolation kit (Miltenyi Biotec), per the manufacturer’s protocol. Cells were grown in RPMI 1640, 10% FCS, 1% v/v Pen-Strep, and 55 μM 2-ME.

ELISA

NP-specific Abs in the sera were determined using ELISA, as previously described (19).

with defective TI-2 Ab responses, owing to decreased or absent B cells in the marginal zone of the spleen or in the serosal cavities (B1 B cells). In contrast to the apparent impact on TI-2 responses, TACI is thought to inhibit some B cell functions. Thus, TACI−/− B cells hyperproliferate in response to polyclonal activators in vitro, and loss of TACI causes autoimmunity in mice (6, 17, 18), suggesting that TACI is a B cell-inhibitory receptor. How TACI combines inhibitory and stimulatory B cell functions is not understood. Here we asked whether TACI regulates B cell differentiation after T-independent stimulation of B cells.

To determine the mechanism by which TACI controls TI-2 Ab responses, we bred TACI-KO mice with the QM mouse (3) to obtain TACI−/− QM mice. Because in QM mice the transgenic H chain was targeted into the JH locus, it can undergo isotype switching and somatic hypermutation. We studied how TACI promotes QM B cell responses to NP-Ficoll (a TI-2 Ag). We found that QM B cells lacking TACI are normally activated by Ag but proliferate longer and have impaired plasma cell differentiation. Our findings suggest that TACI acts remotely from B cell activation and provides a critical signal for cells to exit the cell cycle and to differentiate into plasma cells.

Materials and Methods

Mice and immunizations

TACI-KO and QM mice were previously described (3, 6). TACI-KO mice were bred to QM mice and are in a C57BL/6 (B6) background. Animals were bred and treated according to the policies of Mayo Foundation Institutional Animal Care and Use Committee. Mice were immunized with 30 μg i.p. of NP-Ficoll (NP41-AECM-Ficoll; Biosearch Technologies) diluted in sterile PBS.

Isolation of primary B cells

CD19+ splenocytes were isolated by negative selection with a B cell isolation kit (Miltenyi Biotec), per the manufacturer’s protocol. Cells were grown in RPMI 1640, 10% FCS, 1% v/v Pen-Strep, and 55 μM 2-ME.

ELISA

NP-specific Abs in the sera were determined using ELISA, as previously described (19).
ELISPOT
Multiscreen HTS-HA 96-well plates purchased from Millipore were coated with 5 μg/ml NP-BSA or 5 μg/ml BSA in sodium carbonate buffer overnight at 4°C. Plates were blocked with 5% milk in TBS-Tween, and B cells obtained by negative selection with magnetic Miltenyi microbeads (MACS) were serially diluted from a maximum of 2 × 10⁶ or 5 × 10⁵ cells per well and cultured overnight. ELISPOTS from splenocytes isolated from adoptively transferred mice were not further processed because recipient mice lack T cells and were transferred with isolated B cells. Ab-secreting cells (ASCs) were detected with AP-conjugated goat anti-mouse IgM or IgG Ab (Southern Biotechnologies) for 2 h at 37°C. The plates were developed with 5-bromo-4-chloro-3-indolyl phosphate-NBT substrate (SigmaFast; Sigma-Aldrich), and dots were counted by microscopy.

Thymidine incorporation
Splenocytes or negatively selected B cells isolated using a MACS B cell isolation kit (Milteny Biotec) were plated at concentration 1 × 10⁶ cells/ml and stimulated with NP-Ficoll or LPS at the concentrations shown. At 72 h after the stimulation, 1 μCi of [³H]TdR (MP Biomedicals) per well was added for an additional 16–20 h. At the end of the culture period, 50–60% of cells were viable as determined by trypan blue exclusion. Cells were

FIGURE 3. TACI−/−QM B cells proliferate longer than TACI+/-B cells after immunization. a, DNA content of CD19+ B cells was determined by propidium iodide incorporation followed by fluorescence analysis with a FACSscan flow cytometer and ModFit LT software. Values are the proportion of B cells (CD19+) in the G2-S + M stage of the cell cycle at the time of immunization (basal), at day 4 or at day 6 postimmunization. Results were obtained with QM and QM−TACI-KO mice (each n = 3 for days 4 and 6) immunized with 30 μg of NP-Ficoll and sacrificed at the indicated times. *, Significant differences. b, Spleen frozen sections obtained on day 6 after immunization, stained with anti-GL7 Abs to depict germinal centers (dark brown clusters in the follicle centers). TACI−/− mice have larger and more abundant clusters of GL7+ B cells at the center of the follicles (germinal centers) at day 6 postimmunization and increased numbers of GL7+ B cells 4 and 6 days after T-independent immunization. c and d, Splenocytes were stained for GL7, CD138, and B220. c, Typical flow cytometry plots representing lymphocyte distributions according to B220 and GL7 expression of gated CD138− lymphocytes. Indicated in the diagrams are the gates used to determine the absolute and relative number of GL7+ B cells. d, Number of GL7+ B cells (B220−, GL7+, CD138−) per spleen (left) or the percentage of GL7+ B cells. GL7 positivity was defined with an isotype control. Results are from three independent experiments. e and f, Splenocytes were stained for annexin V, 7-aminoactinomycin D, and B220 to determine the frequency of apoptotic cells (annexin V+, 7-aminoactinomycin D−) after immunization. e, Histograms represent annexin V fluorescence intensity of B220+ cells at days 4 and 6 postimmunization. f, Number of annexin V+ B cells (annexin V+, 7-aminoactinomycin D−, and B220+) expressed as a percentage of annexin V+ B cells. TACI deficiency does not increase the number of apoptotic B cells in the spleen 4 and 6 days after immunization. FSC, Forward scatter.
lysed by hypotonic lysis, transferred to glass fiber filters (Packard), and counted on a Matrix 96-Direct Beta Counter (Packard).

Adoptive transfer

Ten million negatively selected B cells isolated with the B cell isolation kit (Miltenyi Biotech) were injected into the jugular vein of RAG1−/− mice on a B6 background 1 h before immunization.

FACS staining

Biotinylated anti-idiotypic Ab (R2.438.8) directed against the QM Ab (17.2.25 monoclonal) was a gift from Dr. Imanishi-Kari (Tufts University, Boston, MA). All other Abs were purchased from BD Pharmingen. FITC-conjugated mAbs were anti-mouse CD21 (7G6), IgM (AMS9.1), GL7 and B220 (RA3-6B2). PE-conjugated mAbs were anti-mouse CD23 (B384), IgMa (DS-1), CD138 (281-2) and FAS (CD95). Biotinylated mAbs were anti-mouse IgDa (AMS9.1) and CD138 (281-2). Allophycocyanin-conjugated mAbs were anti-mouse CD19 (ID3) and B220 (RA3-6B2). Peanut agglutinin was biotinylated (Vector Laboratories). Biotinylated Abs were revealed by streptavidin-PE-Cy5 purchased from BD Pharmingen. Data were collected using FACSScan or FACSCalibur and analyzed with CellQuest software. For the annexin V binding assay, the cells were first stained with Abs directed to surface Ags and later stained with the Annexin V-PE Apoptosis Detection kit 1 (BD Pharmingen) according to the manufacturer’s recommended protocol. Cell cycle analysis was done according to previously described procedures (20) in isolated B cells.

Results

TACI deficiency compromises IgG TI-2 Ab production and formation of ASCs in QM mice

To explore the involvement of TACI in TI-2 Ab responses, we bred TACI-KO mice (TACI−/−) with QM mice that have enhanced TI-2 responses to NP-Ficoll, in part owing to increased marginal zone NP-specific B cells (4). QM TACI−/− mice had 2.3-fold more plasmocytes and B cells than QM TACI+/+ mice (Fig. 1a). The increase in the number of B cells in QM TACI−/− mice did not alter the proportions of naive (IgM−IgD+, 51% in TACI−/+ vs 43% in TACI−/−), marginal zone (CD21+/hi, CD23+/+, 42% in TACI−/+ vs 44% in TACI−/−) or follicular (CD21+, CD23+/hi; 31% in TACI−/+ vs 32% in TACI−/−) B cells (Fig. 1b).

QM mice respond vigorously to NP-Ficoll, a TI-2 stimulus, by producing NP-specific IgM (3246 μg/ml) and IgG3 (9.0 μg/ml) in the serum 4 days after immunization. QM mice deficient in TACI produced only 1.6 μg/ml NP-specific serum IgG3, 5.6-fold less than TACI+ QM littermates and comparable levels of NP-specific serum IgM (2673 μg/ml), indicating that TACI promotes IgG3 production in response to TI-2 stimuli in QM mice.

Next, we asked whether TACI was required to produce QM ASCs in the spleen after immunization with NP-Ficoll. The number of NP-specific ASCs was determined by ELISPOT 6 days after immunization. TACI−/− QM mice showed 2.0-fold decreased numbers of IgM and 1.6-fold-decreased numbers of IgG NP-specific ASCs per spleen, compared with TACI+ QM mice (Fig. 2a). The number of IgM or IgG ASCs in the bone marrow was also decreased albeit not significantly, by 1.8- and 1.2-fold, respectively, in TACI−/− mice compared with TACI+ QM mice. Analysis of spleen sections showed that the number of B cells that differentiated into plasma cells (CD138+ peaked at day 6 and slowly decreased thereafter (not shown). The number of plasma cells in the perifollicular areas of the spleen was significantly decreased in TACI−/− compared with TACI+ QM mice 6 days after immunization (Fig. 2b). Given that the precursor frequencies of NP-specific B cells were the same in TACI+ QM and TACI−/− QM mice (~85% for both; data not shown), our findings of reduced splenic ASC in QM mice deficient in TACI indicate that TACI promotes terminal differentiation. Decreased numbers of plasma cells were not due to increased apoptosis in TACI−/− QM mice, because TACI−/− CD138+ cells had reduced apoptosis (Fig. 3, d and f), a finding consistent with published reports indicating that an EDAR-TACI fusion induces death in the A20 mouse plasma cell line (17).

TACI deficiency causes B cells to remain proliferating longer and to differentiate less after TI-2 Ag stimulation in vivo

We next tested whether TACI promotes B cell differentiation early or late relative to B cell activation. We first determined whether TACI deficiency enhances QM B cell proliferation by calculating the relative number of CD19+ cells in the G2-S and M stages of the cell cycle after immunization. Fig. 3a shows that 6 days after immunization there were more TACI+ QM B cells cycling (in G2-S or M stages of the cell cycle, 9.6% on average), compared with TACI−/− B cells (5.9% on average). Curiously, the number of TACI+ and TACI−/− B cells cycling 4 days after immunization is similar, suggesting that TACI is necessary at a time remote from activation, thus limiting clonal expansion. Fig. 3a also shows that the number of cycling B cells was greater in nonimmunized mice (2-fold in TACI+ mice and 1.6-fold in TACI−/− mice) than day 6 postimmunization. We speculate that the cycling B cells in the nonimmunized mice are highly selected QM idiotype (Id)+ B cells (19) responding to a variety of environmental Ags producing diverse Ig. In contrast, after immunization with NP-Ficoll Id+ B cells are synchronously stimulated and produce homogeneous Ig, which will give rise to immune complexes that inhibit Id+ B cell activation, decreasing the number of cycling B cells 6 days after immunization.

To determine the extent to which TACI decreases B cell blasts after immunization, we analyzed spleen sections and plasmocytes obtained from TACI+ or TACI−/− QM mice by flow cytometry at days 4 and 6 after immunization. Mouse B cell blasts express an epitope recognized by a rat mAb, GL7. GL7 binds to sialylated glycans, the α-2,6-linked N-acetyllactosaminic acid (Neu5Ac) on lactosamine glycan chains. Neu5Ac expression increases in germinal center B cells owing to repression of CMP-Neu5Ac hydroxylase which converts Neu5Ac to N-glycolylneuraminic acid that is not recognized by GL7. Naito et al. (21) showed recently that
N-glycolyneuraminic acid represses B cell activation and therefore specific suppression of CMP-Neu5Ac hydroxylase enhances activation of germinal center B cells. T-independent stimulation of QM mice generates short-lived germinal centers that mark extensive B cell proliferation (22, 23). The number and size of GL7^{+}/H11001 clusters were increased in TACI^{−}/H11002 mice 6 days after immunization (Fig. 3b). The proportion of follicles with GL7^{+} germinal centers was 88% in TACI^{−} mice and only 51% in TACI^{+} mice 6 days after immunization, indicating enhanced late B cell proliferation in TACI^{−} QM mice (Fig. 3b). Consistently, the number and proportion of B cells (B220-positive) that are blasts (GL7^{+}) were reproducibly increased in the absence of TACI after immunization (Fig. 3, c and d). More than 80% of the GL7^{+} B cells also expressed other germinal center markers such as Fas, bound the peanut agglutinin lectin, and were IgD^{−}. These results suggest that TACI is necessary to stop cycling to allow terminal differentiation.

**TACI deficiency caused impaired plasma cell differentiation owing to a B cell autonomous defect**

To determine whether the impaired Ab production in response to NP-Ficoll was owed to deficiency of TACI on B cells, we performed adoptive transfer experiments. Ten million QM B cells (CD19^{+}) that have or lack TACI were transferred into RAG1^{−/−} recipients (n = 5). At day 6 postimmunization, spleens from recipient mice had 16 \((\pm 3.9) \times 10^6\) or 16 \((\pm 8.9) \times 10^6\) QM TACI^{−} or QM TACI^{+} B cells on average, respectively. Recipients of QM TACI^{−} B cells produced

**FIGURE 5.** TACI \(^{-}\) QM B cells proliferate equally well but differentiate less than TACI\(^{+}\) B cells after stimulation in vitro. \(a\), In vitro activation of QM and QM-TACI-KO B cells was assessed by surface expression of CD69 20 h after stimulation of splenocytes with 1 or 10 \(\mu\)g/ml LPS or NP-Ficoll, as indicated. Similar results were obtained in 4 independent experiments. \(b\) and \(c\), Incorporation of \(\text{[}^3\text{H}\text{TdR}\) by splenocytes or B cells purified from the spleen cultured with LPS (0.1 \(\mu\)g/ml; \(b\)) or NP-Ficoll (0.1 \(\mu\)g/ml; \(c\)), in the concentrations indicated, for 72 h. \(*\), Significant differences. \(d\), Number of syndecan-1\(^{\text{high}},\) IgM\(^{+}\) in 1 million QM or QM-TACI-KO splenocytes, cultured with 1 \(\mu\)g/ml NP-Ficoll, for 72 h. One of three experiments with similar results is shown. \(*\), Significant differences.
Here we show that TACI expressed by B cells has a dual function. In support of this conclusion, we found that although Ag-stimulated B cells lacking TACI were normally activated in response to Ag, they proliferated longer and differentiated poorly into ASCs both in vivo and in vitro. Thus, we conclude that a defect in plasma cell differentiation is the cause for the defective TI-2 Ab production in TACI-KO mice.

TACI−/−QM B cells were activated and proliferated as efficiently as TACI+/+ QM B cells but had impaired plasma cell differentiation after stimulation in vitro

Our findings of severely reduced serum Abs and decreased Ab-secreting cell formation in response to immunization with NP-Ficoll suggested that TACI promotes Ab production. Defective Ab production in TACI−/−QM mice could be owed to defective B cell activation, clonal expansion, or terminal differentiation. To distinguish among these possibilities, we compared activation, proliferation, and differentiation of TACI−/− or TACI+/+ QM B cells in vitro.

TACI−/−QM B cells were activated by LPS or by NP-Ficoll to the same extent and with the same kinetics as TACI+/+ B cells, as indicated by CD69 expression 20 h later (Fig. 5a), indicating that TACI is not required for activation of B cells. TACI−/− splenocytes proliferated more than TACI+/+ splenocytes in response to LPS but not to NP-Ficoll (Fig. 5, b and c, respectively). However, isolated TACI−/− or TACI+/+ B cells proliferated equally in response to LPS (Fig. 5b). Because LPS but not NP-Ficoll stimulates macrophages/microcytes and dendritic cells to produce TACI ligands (24), TACI inhibition of proliferation is apparent only in splenocyte cultures responding to LPS. It is possible that upon LPS stimulation macrophages/microcytes and dendritic cells express glycosaminoglycans that bind APRIL (25), enhancing its stimulatory function.

The inhibitory properties of TACI do not account for the notably decreased Ab responses to TI-2 stimuli. To determine whether TACI promoted plasma cell differentiation, we analyzed expression of syndecan-1 (CD138, a marker of plasma cells) by TACI+/+ or by TACI−/− QM B cells by flow cytometry, 72 h after stimulation. Fig. 5d shows that the number of putative plasma cells (CD138+/hi) was 2-fold lower in cells lacking TACI than in TACI+/+ cells. These results confirmed that TACI is required for efficient plasma cell differentiation.

Discussion

Here we show that TACI expressed by B cells has a dual function. In support of this conclusion, we found that although Ag-stimulated B cells lacking TACI were normally activated in response to Ag, they proliferated longer and differentiated poorly into ASCs both in vivo and in vitro. Thus, we conclude that a defect in plasma cell differentiation is the cause for the defective TI-2 Ab production in TACI-KO mice.

TACI−/−QM mice produced normal levels of NP-specific IgM in response to NP-Ficoll despite severely reduced IgM ASCs. These results suggest that in QM TACI−/− mice, serum IgM is not produced by terminally differentiated B cells (detected in ELISPOT). In fact, Abs can be made by B cells before terminal differentiation in relatively small amounts on a per cell basis (26). Thus, initial IgM Ab production depends greatly on the clonal size. Because we show that TACI−/− QM B cells proliferate to a greater extent and longer than TACI+/+ QM B cells, the clonal size must be larger in the absence of TACI. Therefore, the relatively normal early IgM responses by QM TACI−/− mice may be attributed to Ig secretion by nonplasma cells. Because IgG-producing cells arise later, switched cells are a minority in the clone, and thus the IgG serum level is mostly the product of plasma cells. As TACI promotes differentiation into plasma cells, IgG plasma cells are decreased in TACI−/− relative to TACI+/+ mice, explaining the severely reduced IgG serum levels. It is possible that accelerated proliferation of TACI-KO B cells, in fact, contributes to their decreased ability to differentiate, as is thought to occur in B cells of p18 KO mice (27).

Our results explain how human subjects with common variable immunodeficiency owing to mutations of TACI have specific defects in the production of switched Ig isotypes with an intact class-switching mechanism (28, 29). In these patients, a larger initial clonal size composed mainly of undifferentiated IgM+ B cells would account for the relatively normal IgM levels. In contrast to IgM-producing cells, B cells secreting switched isotypes are much less frequent, and therefore the serum level of switched isotypes is mostly owed to differentiated plasma cells. Thus, excessive expansion and impaired differentiation of B cells in TACI-defective subjects results in switched Ig isotype defects (28, 29).

T-independent activation of B cells induces activation induced cytidine deaminase expression (30), somatic hypermutation (23), and germinal center formation (22), and therefore may potentially originate autoreactive Abs. How the generation of autoreactive Abs is prevented following T-independent stimuli is not known. Assuming that activation induced cytidine deaminase is expressed and the rate of mutation is maintained during clonal expansion, mutants will accumulate in an exponential manner (31). Thus, increased clonal sizes owing to TACI deficiency may increase substantially the number of B cells producing mutated and autoreactive Abs in response to T-independent stimulation. Thus, TACI may limit the development of autoreactive Abs by decreasing clonal expansion and because plasma cells do not mutate, by promoting rapid differentiation. Our results indicate that engagement of TACI promotes termination of proliferation and plasma cell differentiation, limiting the chances of forming autoreactive Abs following T-independent stimulation. This possibility is in agreement with the findings of Grewal and collaborators, who showed increased incidence of autoimmune Abs in aged TACI-KO mice (17).

Acknowledgments

We thank Karen Lien, Lonnie Lindquist, Shari Sutor, and Allan Nilson for technical assistance.

Disclosures

R.J.B. is listed as a coinventor on patents including the TACI gene.

References


