Detection of Antigen-Specific Human Serum Proteins Related to the T-Cell Receptor in Infectious Disease and in an Immune Response to Milk Proteins or Chemicals (44540)

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Abstract. **A monoclonal IgG2 antibody, MG3C9–1 A12, was prepared by immunization of mice with human serum Cohn Fraction III proteins enriched for TCR Ca^+ proteins. MG3C9–1 A12 bound to Mr 28,000, antigen-specific TCR Ca^+, β^-, and TCR Ca^+, β^+ serum proteins associated with TGF-β1, 2. The IgG2 monoclonal antibody also bound to T-lymphocyte proteins but did not bind to B lymphocyte proteins, human albumin, IgM, IgG, IgA, or TGF-β1, 2, 3 immunogenic peptides. Monoclonal MG3C9–1 A12 detected TCR-related proteins specific for filarial extract, milk proteins, or benzoic acid in the sera of individuals with chronic or asymptomatic filariasis, milk intolerance, or sensitivity to toluene, respectively. TCR-related serum proteins were also detected intracellularly in mononuclear cells in frozen sections of ileum from a patient with milk intolerance and reactive mesenteric lymph nodes from a patient with a gastric ulcer. The results suggest that antigen-specific TCR-related serum proteins may be elevated during an immune response to oral, environmental, or infectious stimuli.**

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Extracellular, T-cell–derived proteins that bind specifically to nominal (non-MHC-associated) antigen have been described in T-cell culture fluids (1–11) and murine (12–14), and human (15, 16) serum. These immunoproteins bear T-cell receptor (TCR variable region (15) and α chain constant region (8, 10, 17, 18) epitopes. TCR Ca^+ immunoregulatory proteins have also been described in human ascitic fluid (19). To simplify the nomenclature, we coined the generic term T-cell antigen binding molecule (TABM) to define T-cell proteins that bind specifically to nominal antigen (20). Recently, we reported a rise in serum TABM levels specific for milk proteins or benzoic acid in certain individuals intolerant to milk (21) or sensitive to toluene (16), respectively. Moreover, there was a significant correlation between physical and/or cognitive symptoms and levels of serum TABM specific for the antigen to which the individuals were sensitized.

Soluble, antigen-specific T-cell–derived immunoregulatory factors (7) and TABM share epitopes (10) and are physically and functionally associated with immunoregulatory cytokines (10, 21). We have suggested that TABM may function by antigen-specific focusing of immunoregulatory cytokines such as IL-10 and TGF-β to an area where antigen is deposited (10, 11, 16). Accordingly, measurement of antigen-specific TABM levels could provide insight into the

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type (Th1, Th2) and strength of a T-cell response to antigen after immunization or during disease. Moreover, perhaps a high local concentration of cytokine-bearing TABM at an antigenic site causes a particular set of symptoms due to the cytokine. In this report, we have developed and used a monoclonal antibody to human TABM to quantitate TABM specific for a sensitizing antigen in filariasis, milk intolerance, or sensitivity to an environmental chemical. The results confirm and extend our observations that a serum TABM response indicates a Th2-type immune response that may be associated with downregulation of a cell-mediated immune response.

Materials and Methods

Antisera/Antibodies. Monoclonal antibodies to TCR alpha (TCRα-1) and beta chains (TCRβ-1) were purchased from T Cell Diagnostics (Woburn, MA) and T Cell Sciences (Cambridge, MA), respectively. Rabbit antibodies to TGF-β1, TGF-β2, or TGF-β3 immunogenic peptides were purchased from Santa Cruz Immunobiologicals (Santa Cruz, CA).

Monoclonal antihuman TABM MG3C9-1A12 was prepared by intraperitoneal immunization of BALB/c mice with 5–10 μg of human TABM (hTABM) purified from Cohn Fraction III proteins (a kind gift from Dr. W. Landesberg, Baxter Pharmaceuticals, Deerfield, IL) and Freund's complete adjuvant. The mice received a second intraperitoneal injection of hTABM in Freund's incomplete adjuvant 1 month later. Three and seven months later, the mice received intraperitoneal injections of hTABM in 300 μg of polyadenylic:polyuridylic (poly A:U) acid (Boehringer, Kankakee, IL). Four days before fusion, a fifth intraperitoneal injection of hTABM + poly (A:U) was given. Murine spleen cells were fused with the murine NS-1 myeloma cell line.

Subjects. *Chemical sensitivity.* Serum from a patient with a history of sensitivity to toluene and a high titer of TABM specific for benzoic acid (BA) (16) was used as a source of BA-specific TABM.

Filariasis. Sera from patients with chronic, acute filariasis, asymptomatic with microfilaremia or cerebral malaria have been described (22). Patients from Orissa, India, were categorized after clinical and/or parasitological examination (22) as: (i) acute filariasis (presenting with adenolymphangitis with or without fever, lymphedema); (ii) chronic filariasis (persistent lymphedema and/or hydrocoele with no history of adenolymphangitis in the last three months); (iii) asymptomatic microfilariae carriers (asymptomatic individuals identified by microscopic examination of nocturnal blood in Giemsa-stained thick smear). Patients with noncerebral or cerebral malaria were chosen for control sera.

Milk intolerance. A 47-year-old female with a 10-year history of milk protein intolerance presented as a triad of recurrent uveitis, diffuse small joint arthralgias and abdominal pain with occasional diarrhea. This patient has no concurrent history of other autoimmune disorders, other food allergies, or inflammatory bowel diseases, including celiac sprue and Crohn's disease. Positive risk factors for any bowel disorder included frequent travel to developing countries, but the stools were negative for ova and parasites. The history is negative for excessive use of nonsteroidal anti-inflammatory drugs. Laboratory testing was normal for anemia and stigmata of inflammation although she had a mild elevation of the rheumatoid factor titer. The physical examination was normal and was specifically negative for abdominal findings, and small and large joint changes. Ocular examination revealed recurrent anterior uveitis. Colonoscopy with intubation of the ileum revealed a normal appearance for both colonic and small bowel mucous. Colonic biopsies were normal, but the ileal sections revealed a mild increase in lamina propria lymphocytes and plasma cells. The specimens were taken for diagnostic purposes at a time of milk protein avoidance and minimal clinical symptoms.

Proteins/Antigens. *Immunoglobulin-depleted serum.* Immunoglobulin (Ig)-depleted serum was purchased from Sigma (St. Louis, MO).

TABM-enriched Cohn Fraction III proteins. Five grams of human serum Cohn Fraction III proteins were mixed with 10 ml 2 *M* urea in phosphate-buffered saline (PBS, pH 7.2), and after 15 min agitation, the mixture was centrifuged for 30 min at 9000 rpm. The supernatant was precipitated by the addition of $(NH_4)_2SO_4$ to 43%, and the precipitate formed at 4°C for 2 hr. The precipitate was recovered by centrifugation at 9000 rpm for 30 min and solubilized in 10 ml 2 *M* urea/PBS. The solubilized precipitate was centrifuged at 9000 rpm for 30 min, and the supernatant loaded on a 3.5 cm × 67 cm column containing sephacryl S-300 equilibrated in the same buffer used to solubilize the precipitate. The column was pumped at 950 ml/ hr, and 5-ml column fractions were collected. Based on the predetermined chromatographic positions of IgM, IgG, human albumin and ovalbumin, the column fractions were pooled as Fraction I, void volume (> MW 600,000), Fraction II (MW 10^5–3×10^5), Fraction III (MW 4×10^4–1×10^5), Fraction IV (MW $< 3 \times 10^4$). Column fractions I-IV were coated (500 ng/well) to wells of microtiter trays (Nunc, Costar High Binding Fisher, Atlanta, GA). Each column fraction was assayed (by ELISA) for TABM by reaction with rabbit anti-TABM (15) antiserum. Fraction I containing TABM was precipitated by the addition of $(NH_4)_2SO_4$ to a final concentration of 43%, as described (21). The precipitated proteins were solubilized in PBS and then absorbed with 1 ml sepharose 4B beads conjugated with antihuman Ig (1 mg/ml whole molecule, Sigma) and antihuman albumin (Sigma, St. Louis, MO).

Antigens. β-Lactoglobulin (BLG), α-S-casein (CA) and α-lactalbumin were purchased from Sigma Chemical Co. (St. Louis, MO). A microfilarial extract of *Microfilarium digitata* was prepared by grinding ≈ 50 lyophilized M. digitata in phosphate-buffered saline (PBS, pH 7.2) as described (22). Debris was removed by centrifugation at 2500 rpm for 20 min, and the extract stored at –70°C.

Enzyme-Linked Immunosorbent Assay (ELISA). Most ELISAs were duplicated in laboratories in Australia and the United States. Generally, microtiter trays were coated with 100–1000 ng/well protein and blocked with 200 μl 0.1% gelatin. A dilution of primary antibody in wash buffer was incubated for 1 hr–1.5 hr at 37°C, and after washing, alkaline phosphatase or glucose oxidase-conjugated goat antirabbit or mouse Ig (whole molecule), antibody (Sigma) was added to the microtiter trays for 1 hr–1.5 hr at 37°C or 1 hr at ambient temperature, 1 hr at 37°C. Bound antibody was visualized with p-nitrophenyl phosphate or tetramethyl benzidine substrate.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate (SDS-PAGE). Proteins were mixed 1:1 with SDS-PAGE sample buffer ± 5% β_2-mercaptoethanol and boiled 5 min. The proteins were then resolved in 8%–25% or 10%–15% polyacrylamide gradient gels using the PHAST (Pharmacia) system. Electrophoresed proteins were silver-stained for increased sensitivity. Molecular weights were determined by the mobilities of prestained proteins (Biorad).

Immunoblotting. Proteins resolved by SDS-PAGE were electrophoretically transferred to a polyvinylpyrollidone (immobilon, Millipore) membrane. The membrane was blocked by incubation in 10 ml of 5% powdered milk for 2 hr at 37°C. The membrane was washed and then incubated overnight at 4°C with 10 ml of 1:1000 monoclonal M3C9–1 A12 (anti-TABM) antibodies in wash buffer (10). Bound antibodies were detected by the addition of 10 ml of alkaline phosphatase-conjugated goat anti-murine IgG (human IgG absorbed) antibodies and incubation at 4°C for 2 hr. The membrane was then washed for 1 hr, and CSPD chemoluminescent substrate (Boehringer-Mannheim, Indianapolis, IN) was added to the membrane according to the manufacturer's instructions. Kodak x-ray film was overlaid on the membrane, and the exposure was held for 1 hr at ambient temperature, 1 hr at 37°C. Following exposure, the film was developed automatically.

Immunohistopathology. Frozen sections of an ileum biopsy or mesenteric lymph node were fixed in 4% paraformaldehyde for 15 min. The sections were washed in saponin/PBS and held in 0.3% H_2O_2 in saponin/PBS for 30 min. After one wash in saponin/PBS, 5% horse serum with 1:5–1:50 dilutions of MG3C9–1A12 was added, and the sections were held overnight. The sections were washed with PBS after incubation with MG3C9–1A12 and biotin-conjugated horse anti-mouse IgG (1:200) added. After a wash with PBS, an avidin biotin peroxidase complex was added, and after a PBS wash, developed with aminoethyl carbazole (AEC), and counterstained with hemotoxylin.

Results

Monoclonal Antibody MG3C9-1A12 Is Specific for Proteins in Human Serum and Extracts of T Lymphocytes. The specificity of monoclonal antibody MG3C9-1A12 was defined by the binding of the antibody to Cohn Fraction III proteins enriched for TABM (Figs. 1A & 1B), human serum or immunoglobulin-depleted human serum (Fig. 1C). MG3C9-1A12 bound strongly to 100 ng Cohn Fraction III proteins that were not bound by antibodies to human immunoglobulin κ or λ chains or albumin (Fig. 1A). These Cohn Fraction III proteins diluted up to 1:30,000 (10 ng) were also bound by antibodies to TCR Cα chains. In contrast, anti-H-2κ (control) antibodies and anti-TCR Cβ bound to only 150 μg of the Cohn Fraction III proteins (Fig. 1B).

Immunoblots of reduced serum and Cohn Fraction III proteins with MG3C9-1A12 demonstrated only an Mr 28,000 molecular species (Fig. 2). To determine the reaction of monoclonal MG3C9-1A12 with T and/or B lymphocyte proteins, Jurkat (T) and A-1 (B) cells were lysed with Triton X-100, and the lysates mixed with MG3C9-1A12 sepharose beads. After incubation, the beads were washed and eluted with 0.2 *M* $NaCO_3$. Aliquots of the eluate were coated to microtiter trays, and as shown in Figure 3, MG3C9-1A12 detected an antigen in lysates of Jurkat cells, but not in A-1 (B) cells. In addition, MG3C9-1A12 does not bind human IgG, IgM, IgA, or albumin in ELISA (data not shown).

Monoclonal 3C9-1A12 Detects TCR α^+, β^- Antigen-Binding Serum Proteins. To determine the antigen-binding capacity of serum proteins "recognized" by monoclonal MG3C9-1A12, serum from a patient with a high titer of TABM specific for benzoic acid (16) was absorbed to sepharose beads conjugated with monoclonal 3C9-1A12. The beads were washed and then eluted with pH 2.8 glycine. Serum molecules adsorbed to and eluted from MG3C9-1A12 were adsorbed to and eluted from sepharose beads conjugated with benzoic acid–human serum albumin (BA-HSA). Proteins eluted from MG3C9-1A12 and effluent and MGC39-1A12$^+$ proteins eluted from BA-sepharose were coated to microtiter trays for ELISA with antibodies to TCR constant region epitopes (Fig. 4A). MG3C9-1A12–eluted proteins and Cohn Fraction III, MG3C9-1 A12$^+$ proteins bore an epitope detected by antibodies to TCR Cα (Figs. 3A & 3B). Antibodies to TCR Cβ bound total M3C9-1A12–eluted proteins at lower intensity than anti-TCR Cα antibodies. BA-binding, MG3C9-1A12$^+$ proteins were detected by anti-TCR Cα, but not Cβ or Cδ antibodies (data not shown). However, MG3C9-1A12$^+$ proteins that did not bind to BA-HSA-sepharose were detected equally by anti-TCR Cα and anti-TCR Cβ antibodies. The anti-TCR antibodies or MG3C9-1A12 did not bind to human albumin (data not shown).

To determine further the antigen-binding specificity of the MG3C9$^+$, benzoic acid binding proteins (BA-TABM), the proteins were added to microtiter wells coated with trinitrophenol-human serum albumin (TNP-HSA), bovine serum albumin (BSA), or human serum albumin (HSA). After incubation and washing, MG3C9-1A12 was added to the plates followed by alkaline-phosphatase-conjugated goat anti-mouse IgG. As shown in Figure 4B, MG3C9-1A12 detected BA-TABM binding to TNP-HSA, but not BSA or

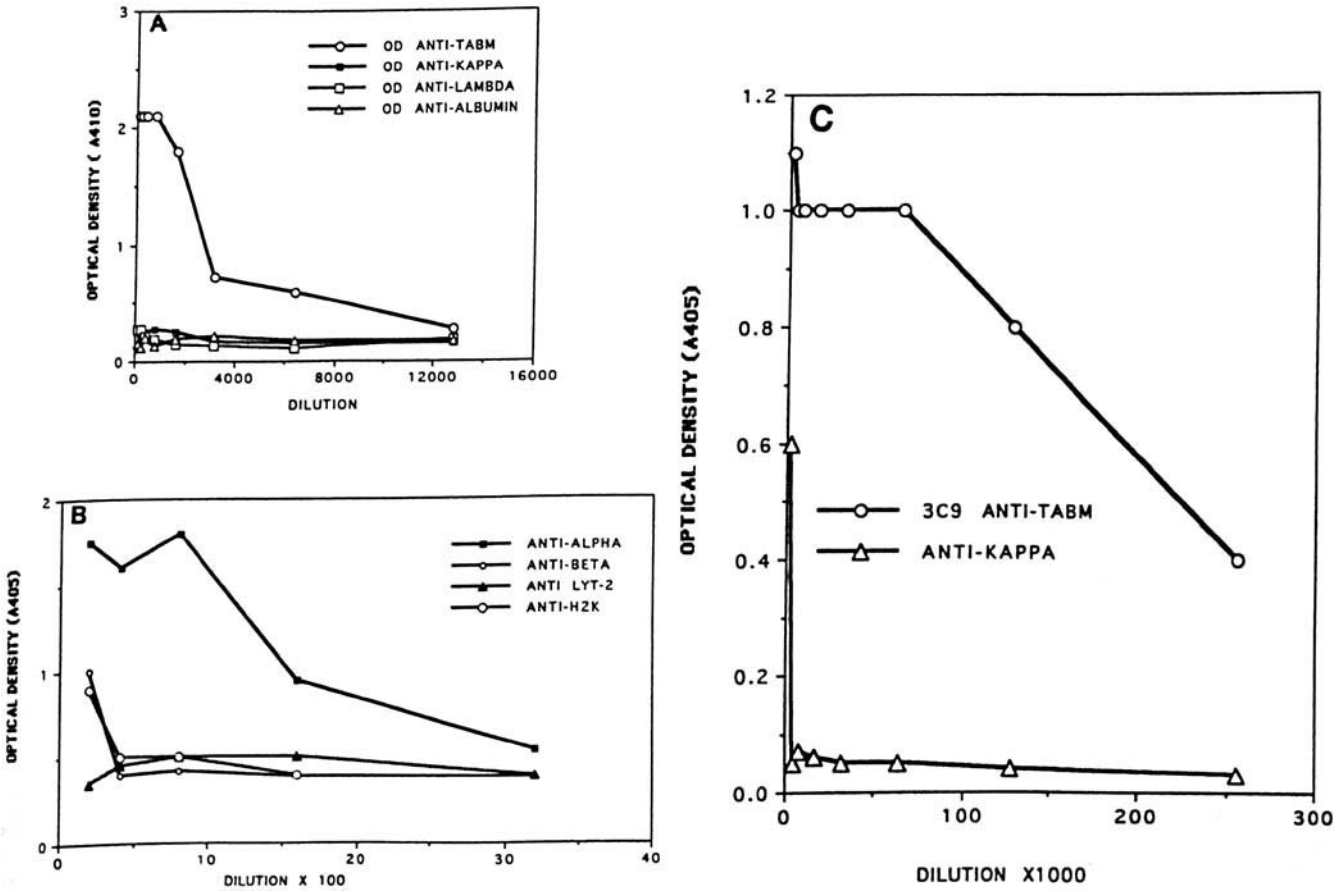


Figure 1. Monoclonal antibody MG3C9-1A12 binds TABM. (A) Microtiter trays were coated with 500 ng of Cohn Fraction III TABM, and 100 μl of MG3C9-1A12 (1:1000), monoclonal anti-κ, anti-λ, or rabbit antihuman albumin was added to the wells. Bound antibodies were developed with alkaline phosphatase-conjugated goat anti-rabbit or mouse IgG. (B) Microtiter trays were coated with 7.8–500 ng Cohn Fraction III TABM and 100 μl 1:20 anti-TCR Cα (anti-α), anti-TCR Cβ (anti-β), anti-Lyt2 or H-2κ was added. Bound antibodies were developed as in Figure 1A. (C) One hundred μl of dilutions of immunoglobulin-depleted serum were coated to microtiter wells. MG3C9-1A12 (1:1000) was added to the wells and after washing, alkaline-phosphatase-conjugated goat anti-mouse IgG was added. After washing, p-nitrophenyl phosphate substrate was added, and optical density was determined after 5 min of incubation.

HSA. Elsewhere (16, 23), we show that BA TABM binds to BA-HSA, BA, and DNP.

Detection and Quantitation of TABM Specific for Filarial Antigens in the Sera of Proteins Infested with Filarial Worms. To quantitate TABM production in an infectious disease, the sera of asymptomatic, microfilaremic individuals or individuals with acute or chronic filariasis, were assayed for TABM and immunoglobulins that bind to antigens of *M. digitata*. As shown in Figure 5, asymptomatic/microfilaremic individuals and individuals with chronic filariasis had a high titer of TABM binding to *M. digitata* in ELISA, whereas individuals with acute filariasis did not have TABM binding to *M. digitata* extract. Sera containing antibodies or TABM to *M. digitata* did not contain antibodies to α-lactalbumin, casein, bovine, or human albumin (data not shown). In addition, we did not detect anti-*M. digitata* TABM in the (control) sera of individuals with cerebral or noncerebral malaria or healthy individuals. Asymptomatic/microfilaremic individuals also had a high anti-*M. digitata* immunoglobulin titer as did individuals with chronic or acute filariasis, although at a lower titer. Individuals with cerebral or noncerebral malaria or serum from healthy (U.S.) controls had anti-*M. digitata* immunoglobulin at titers well below that of individuals infested with filarial worms.

MG3C9-1A12 Detects TABM Specific for Milk Proteins in the Serum of an Individual Intolerant to Cow's Milk. Previously we used polyclonal anti-TABM antisera to quantitate TABM specific for milk proteins in the sera of individuals intolerant to milk (21). To confirm and extend this observation, we titrated serum from an individual intolerant to milk on microtiter trays coated with α-lactalbumin, β-lactoglobulin, or casein. Monoclonal MG3C9-1A12 detected TABM binding to α-lactalbumin and casein with titers of 1:640 and 1:160, respectively (Fig. 6A), whereas control sera from healthy individuals or an individual with cerebral malaria had TABM titers < 1:64 to milk proteins. The patient had significant immunoglobulin titers to both α-lactoglobin and casein (Fig. 6B). As shown in Figure 7A, MG3C9-1A12 stained mononuclear cells in a frozen section of an ileum biopsy from the milk-intolerant patient. MG3C9-1A12 also stained cells in the outer area of

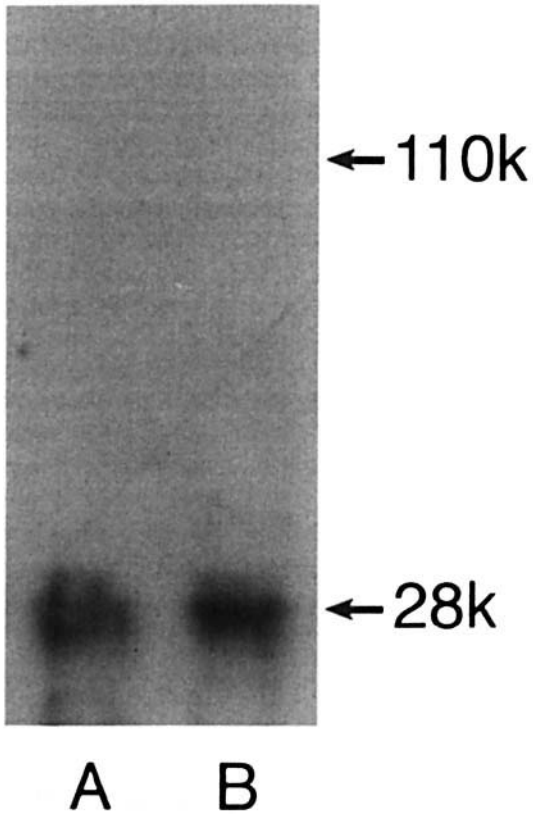


Figure 2. Immunoblotting of TABM and serum with monoclonal MG3C9-1A12. Two hundred fifty µl of a 1:50 dilution of normal human serum and 15 µg of Cohn Fraction III TABM were reduced with 5% β2-mercaptoethanol, and 4 µl of a 1:50 dilution of serum (≈ 5600 ng) or 240 ng purified TABM were resolved in an 8%–25% polyacrylamide gel. Proteins in the electrophoresed gel were transferred to an immobilon membrane and blotted with 10 ml of a 1:1000 dilution of protein G-purified monoclonal MG3C9-1A12. Molecular weights were determined by the mobilities of prestained standard proteins. (A) Cohn Fraction III TABM, (B) human serum.

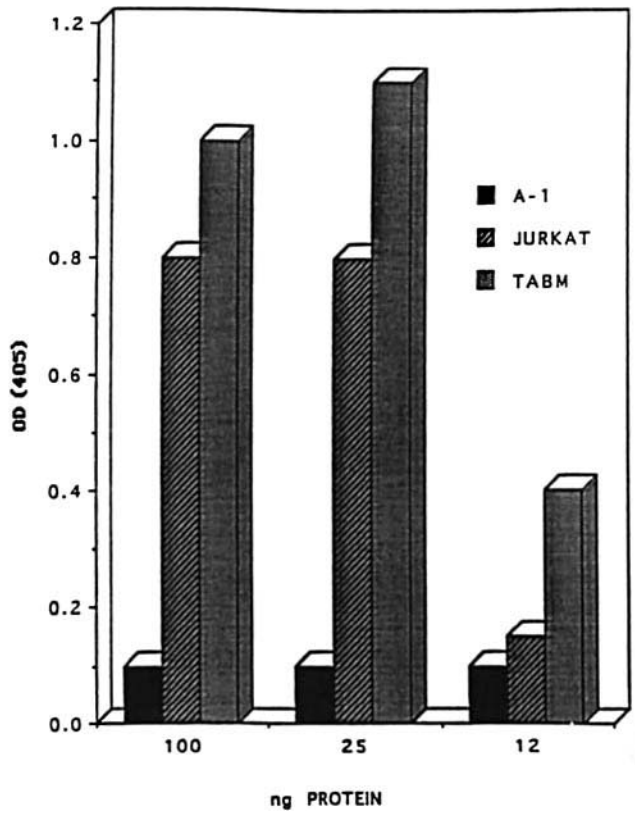


Figure 3. MG3C9–1A12 detects T cell, but not B cell proteins. In this experiment, 100–200 ng Jurkat (T), A1 (B) lysates, or Cohn Fraction III proteins (TABM) were coated to microtiter trays. One hundred µl of a 1:1000 dilution of MG3C9-1A12 was added to the trays and binding of MG3C9-1A12 antibodies was detected by alkaline phosphatase-goat anti-mouse IgG and p-nitrophenylphosphate substrate.

germinal centers of reactive mesenteric lymph node biopsies from a patient with a gastric ulcer (Fig. 7B). The staining is consistent with cytoplasmic staining, and fixed sections were not stained. Moreover, MGC39-1A12 did not bind to the cell surface of peripheral blood lymphocytes.

Discussion

Soluble, antigen-specific T-cell–derived proteins that are recognized by antibodies to TCR Cα epitopes have had an extensive history in basic immunology (11, 24). These immunoproteins mostly have been shown to be affector or effector molecules in the downregulation of cell-mediated immunity (24). In mice, TABM or antigen-specific TCR $C\alpha^+$ "suppressor factors" have been described in serum when the mice are immunized to modulate cell-mediated immunity (12, 25, 26). Generally, antigen-specific TABM levels rise in serum during a Th2-type response (14, 21). Accordingly, quantitation of serum TABM levels could provide some insight into the type and bias of an immune response.

In ELISA, monoclonal MG3C9-1A12 identified TABM in the serum of a healthy individual and serum depleted of immunoglobulins. Other ELISAs demonstrated that this antibody does not bind to purified human IgG, IgM, IgA, or albumin, but does bind to the Mr 28,000 Cohn Fraction III, TCR $C\alpha^+$ proteins used to prepare MG3C9-1A12. The lower reaction of anti-H2K and anti-TCR Cβ with only 500 ng Cohn Fraction II proteins indicates that this fraction has a small amount of human serum proteins that cross-react with murine H2K or human TCR Cβ. Since this reaction did not occur after dilution of the proteins to 250 ng, the reaction at 500 ng may be an artifact. These Cohn Fraction III proteins represent <0.02% of the proteins in serum. The immunoblots of reduced serum and purified TABM were similar (Mr 28,000). The somewhat greater intensity of the blot of serum as compared with purified TABM may be due to more TABM in the serum sample (>240 ng) or more native TABM in the serum. Although MG3C9-1A12 does not stain the cell membranes of viable lymphocytes, we did detect cytoplasmic staining of cells in the ileum of an individual with milk tolerance and lymphoid cells in the outer area of germinal centers in reactive mesenteric lymph nodes. $CD4^+$ T cells are located in the outer areas of germinal centers (27–29), and because MG3C9-1A12 is specific for a T-lymphocyte protein, it is probable that the

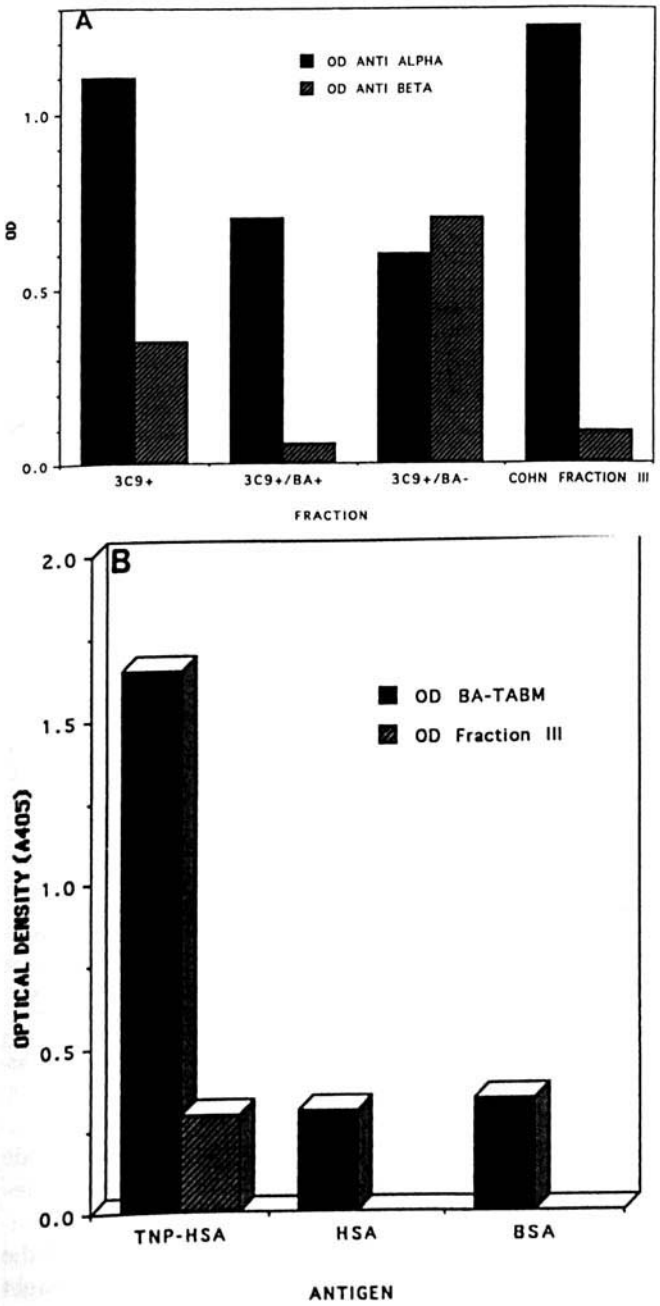


Figure 4. BA-specific, MG3C9-1A12$^+$ TABM bear TCRα epitopes. Serum from a patient with a high titer of TABM to BA-HSA was absorbed to MG3C9-1A12–sepharose beads. The beads were eluted, and the eluted proteins adsorbed to and eluted from BA-HSA-sepharose beads. (A) Two hundred ng total MG3C9-1A12–eluted proteins, BA-HSA–eluted proteins, nonbinding (BA-HSA effluent) proteins, and Cohn Fraction III proteins were coated to microtiter wells and anti-TCR Cα-1 and Cβ-1 were added to the wells. Bound antibody was detected with alkaline phosphatase-conjugated goat anti-mouse IgG. (B) One hundred μl (1 μg) of MG3C9-1A12$^+$ BA-specific proteins or Cohn Fraction III were added to microtiter wells coated with 1 μg/well TNP-HSA, BSA, or HSA. After 1.5 hr incubation at 37°C, the plates were washed, and 100 μl of a 1:1000 dilution of MG3C9-1A12 was added to the plates. Bound antibody was detected with alkaline-phosphatase-conjugated goat anti-mouse IgG.

MG3C9-1A12$^+$ cells in the lymph node are CD4$^+$ germinal center T cells.

Monoclonal MG3C9-1A12 identified TABM specific

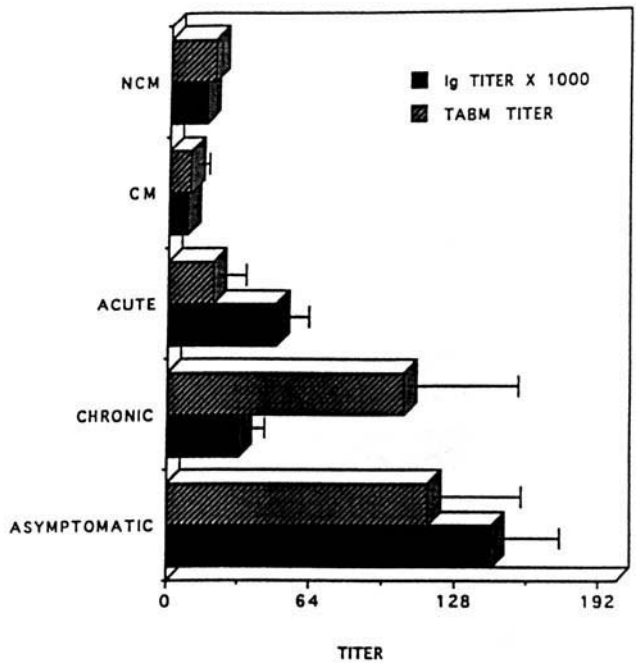
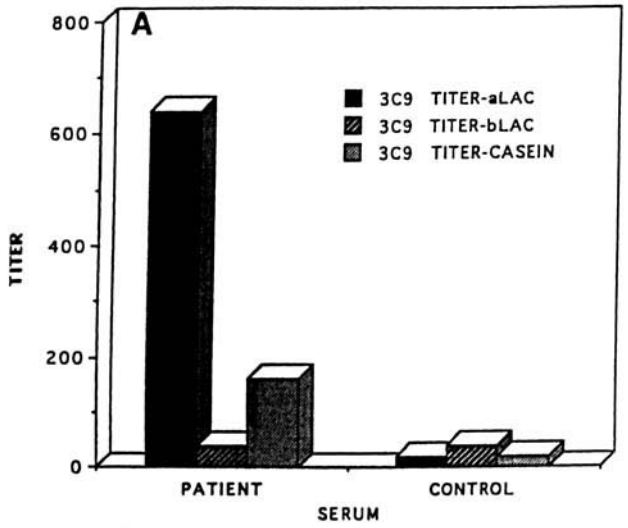


Figure 5. Quantitation of M. digitata-specific TABM in filariasis. Microtiter trays were coated with 1 μg/well of an extract of M. digitata. One hundred μl of dilutions of antisera were added and after washing, MG3C9-1A12 or monoclonal anti-κ antibody was added. After washing, bound antibody was detected with alkaline phosphatase-conjugated goat anti-mouse IgG and p-nitrophenyl phosphate. ASYMPTOMATIC: sera from microfilaria-infested, asymptomatic individuals; CHRONIC: sera from individuals with chronic filariasis; ACUTE: serum from individuals with acute filariasis; CM: sera from individuals with cerebral malaria; NCM: sera from individuals with noncerebral malaria. Data represent the mean titer ± SE of four to eight sera/group.

for BA-HSA in the serum of a patient with a high titer of TABM to this antigen. Like the immunogen used to produce MG3C9-1A12, the serum proteins adsorbed to and eluted from MG3C9-1A12 sepharose and BA-HSA-sepharose bear TCR Cα, but not TCR Cβ (or Cδ) epitopes. No TCR Cβ epitopes were detected in the (immunogen) Cohn Fraction III proteins, and the detection of TCR Cβ epitopes in total MG3C9-1A12$^+$ serum proteins was weak (Fig. 3A). BA-binding TABMs were TCR Cα$^+$ and β$^-$, whereas nonbinding serum TABMs were TCR Cα$^+$ and β$^+$. It is tempting to speculate that the associated β chain (or a molecule sharing an epitope with a TCR β-chain) impedes the binding of unprocessed antigen by the TCR Cα$^+$ protein. These MG3C9-1A12$^+$ proteins are specific for benzoic acid as detected by ELISA for binding to BA-HSA, but not human or bovine serum albumin or the competitive binding of soluble BA-HSA (16). Because the Mr 28,000 human serum TABMs bear TCR Cα epitopes, it is probable that the serum TABMs are T-cell–derived. In support of this contention, MG3C9-1A12 binds to proteins from a T-cell line, but does not bind to B-cell proteins, serum albumin, immunoglobulins, or TGF-β. Although TABMs detected by MG3C9-1A12 are recognized by anti-TCR Cα antibodies, TCRα chains are probably not recognized by MG3C9-1A12. Unlike anti-TCR antibodies, MG3C9-1A12 binds to cytoplas-



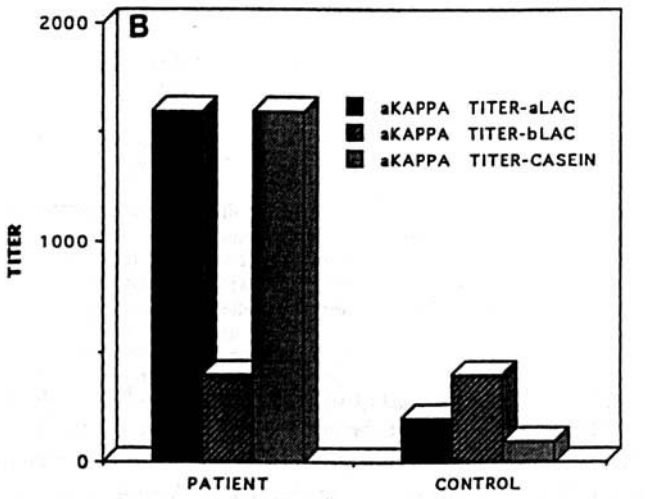


Figure 6. Detection of TABM and immunoglobulins specific for milk proteins in the serum of a milk-intolerant patient. Microtiter trays were coated with 1 μg/well α-lactoalbumin (aLAC), β-lactoglobulin (bLAC), or casein. Serum from a milk-intolerant patient or (control) serum from a patient with noncerebral malaria was added and after incubation and washing, (A) MG3C9-1A12 or (B) monoclonal anti-κ antibody was added. Bound antibody was detected as in Figure 5.

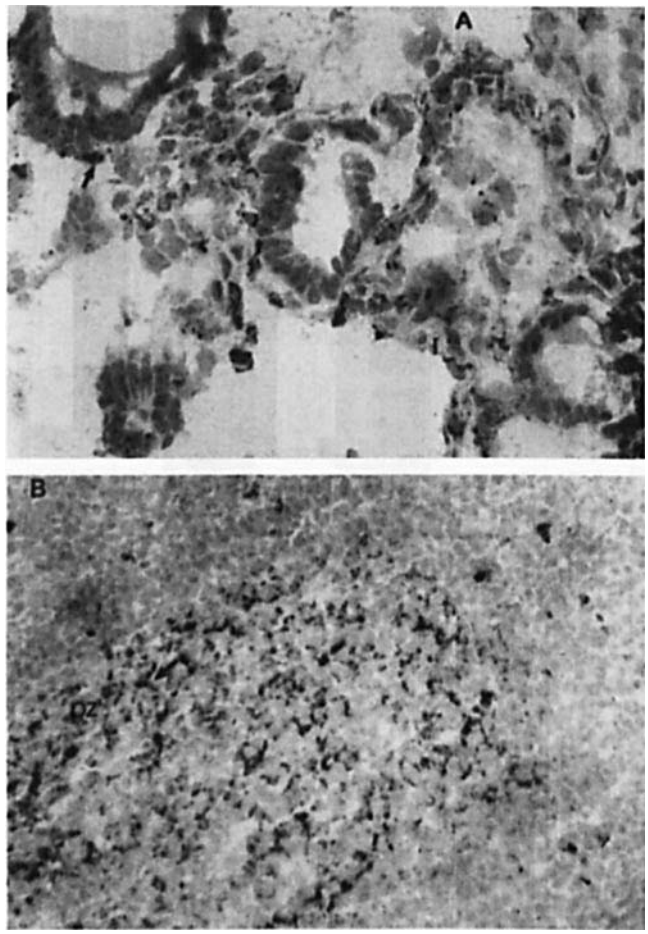


Figure 7. Detection of MG3C9-1A12$^+$ cells in frozen sections of reactive lymph nodes and ileum. (A) A frozen section of an ileum biopsy from a patient with milk intolerance and (B) reactive mesenteric lymph nodes from a patient with a gastric ulcer were stained with MG3C9-1A12 and biotin:avidin–conjugated complexes. The arrow indicates a stained cell. OZ: follicular outer zone.

mic, but not cell-surface constituents. It is unlikely that the association of a TCRβ chain would sterically obscure a TCR Cα epitope detected by MG3C9-1A12 because some MG3C9-1A12$^+$ serum molecules are recognized by both anti-TCR Cα and anti-TCR Cβ. However, TCR Cα chains could share an epitope with TABM recognized by MG3C9-1A12 (Fig. 3). Murine TABMs share an epitope with TCR Cα, but the amino acid sequence of an amino terminal peptide and a tryptic peptide demonstrate that murine TABMs are similar, but not identical, to TCRα chains (24). In aggregate, MG3C9-1A12 recognized a T-cell–derived, TCR Cα$^+$ protein that binds nonprocessed antigen. Although it is unlikely that MG3C9-1A12 is specific for a TCR Cα epitope, the determination of the amino acid and/or nucleotide sequence of TABM or ELISA of MG3C9-1A12 with a recombinant TCRα chain should answer this question. Identification of the (presumed) amino acid sequence of the epitope recognized by MG3C9-1A12 would provide insight into the expression of this epitope by other proteins. Experiments directed to these ends are in progress.

Molecular sieving of Cohn Fraction III proteins detected by MG3C9-1A12 suggests that the Mr 28,000 polypeptides detected by MG3C9-1A12 may be a "protomer" of a large (>10^6) complex (Fey G, Cone RE, unpublished observations). Although TABMs tend to oligomerize (11, 24), the large molecular size of TABMs may also be due to an association of TABMs with another molecular species. Interleukin-10 has been shown to be associated noncovalently with TABMs produced by antigen-specific murine T-cell hybrids (10), and human serum TABM specific for β-lactoglobulin associated noncovalently with TGF-β1 and 2 have been described (21). Moreover, the TCRα$^+$ Cohn Fraction III proteins used to prepare MG3C9-1A12 are associated with TGF-β1 and TGF-β2 (Cone RE, unpublished

data). The amount of TGF-β detected in association with BA-TABM is increased when the TABM bound specifically to optimal proportions of benzoic acid. This antigenic activation of TGF-β associated with TABM correlates with an antigen-specific functional activation of TABM-associated TGF-β (23) when BA-TABMs bind to antigen. These observations suggest that TGF-β associated with TABMs becomes active only within the vicinity of optimal amounts of antigen, thereby regulating the immune response to that antigen. Others have also reported soluble TCR $C\alpha^+$ proteins associated with immunoregulatory cytokines (9, 19, 30).

The association of TABMs with immunoregulatory cytokines provides support for our suggestion (10, 11, 21) that TABMs function as a soluble, antigen-specific element that focuses immunoregulatory cytokines. It is notable that individuals with filariasis that are asymptomatic, but microfilaremic, or individuals with chronic filariasis have high antifilarial TABM titers whereas individuals with acute filariasis have low antifilarial TABM titers. The acute disease is associated with a Th1-type response that may serve to eliminate the antigen (31, 32). Because TGF-β and/or IL-10 suppress immune removal of microfilariae (33), perhaps TABMs associated with immunoregulatory cytokines downregulate a Th-1 response to the microfilariae. Circulating TGF-β levels also appear to be associated with the regulation of the immune response to murine malaria (34), murine leishmaniasis (35), and a murine response to Schistosoma mansoni (36). Accordingly, the detection of circulating TABMs should indicate whether a regulatory T-cell response has been initiated during infection or vaccination. We have observed that TGF-β associated with TABMs enhances the release of substance P by sensory nerves (23). Since the specific interaction of TABM with antigen activates (releases?) associated TGF-β, collateral effects of the cytokines such as TGF-β may be responsible for certain manifestations of an immune-mediated condition (16). Thus, the identification and quantitation of serum TABM may be useful to evaluate infectious disease, disorders of the immune system, and the efficacy of vaccines.

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1. Ptak W, Askenase PW, Rosenstein RW, Gershon RK. Transfer of an antigen-specific immediate hypersensitivity-like reaction with an antigen-binding factor produced by T cells. Proc Natl Acad Sci U S A **79**:1969–1974, 1982.
2. Steele JK, Chu NR, Chan AR, North DG, Levy J. Isolation of an antigen-specific T suppressor factor that suppresses the *in vivo* response of DBA/J mice to ferredoxin. J Immunol **19**:469–477, 1986.
3. Guy R, Ulrich SJ, Foo-Philips M, Hathcock KS, Apella E, Hodes RJ. Antigen-specific helper function of cell-free T-cell products bearing TcR V-β determinants. Science **204**:1477–1479, 1989.
4. Iwata M, Katarnwak M, Kubo R, Ishizaka K. Relationship between T-cell receptors and antigen-binding factors. J Immunol **143**:3909–3916, 1989.
5. Bristow C, Marchalonis JJ. Solid phase antigen binding by purified immunoproteins from antigen-specific T-cell hybridomas. Mol Immunol **26**:611–624, 1989.
6. Kuchroo K, Steele JK, O'Hara R Jr., Jayaraman S, Selvra P, Greenfield E, Kubo R, Dorf ME. Relationships between antigen-specific helper and inducer T-cell hybridomas. J Immunol **145**:438–448, 1990.
7. Malley A, Zaleny-Pooley M, Murray G. Stabilization and characterization of antigen-specific T suppressor inducer and T suppressor effector molecules. J Immunol Methods **178**:321–339, 1995.
8. Onda P, Bruner T, Messier H, Bissonette R, Echevarri F, Baier G, Fotedar A, Green DR. Immunoregulatory activity of a T-cell receptor α-chain demonstrated by *in vitro* transplantation and transcription. Proc Natl Acad Sci U S A **92**:3004–3008, 1995.
9. Ishii Y, Nakano T, Ishizaka K. Biochemical characterization of an antigen-specific glycosylation inhibition factor from antigen-specific suppressor T cells. J Immunol **156**:1735–1742, 1996.
10. Cone RE, Wang Y, O'Rourke J, Malley A. Extracellular soluble antigen-specific T-cell proteins related to the T-cell proteins related to the T-cell receptor for antigen (sTCRr): Serologic and primary amino acid sequence similarity to T-cell receptor α-chains and association with cytokines. J Interferon Cytokine Res **18**:55–67, 1998.
11. Cone RE. Soluble extracellular antigen-specific T-cell immunoproteins. J Leuk Biol **59**:605–612, 1996.
12. Ferguson TA, Iverson GM. Isolation and characterization of an antigen-specific suppressor-inducer molecule from serum of hyperimmune mice using monoclonal antibody. J Immunol **137**:3896–3902, 1986.
13. Van Loveren H, Ratzlaff RE, Kato K, Meade R, Ferguson T, Iverson GM, Janeway CH, Askenase P. Immune serum from mice contact sensitized with picryl chloride contains an antigen-specific T-cell factor that transfers immediate cutaneous reactivity. Eur J Immunol **16**:1203–1211, 1986.
14. Urbanski M, Cone RE. T-cell–derived, antigen-specific humoral immune response. II. Further characterization of the response and the antigen binding T-cell immunoproteins. Cell Immunol **153**:131–141, 1994.
15. Dibrino M, Ravindran B, Cone RE. T-cell–derived proteins from normal human sera and their relationship to T-cell antigen-binding molecules. Clin Immunol Immunopathol **59**:271–287, 1991.
16. Little CH, Georgiou G, Shelton MJ, Simpson FS, Cone RE. Clinical and immunological responses in subjects sensitive to solvents. Arch Environ Health **54**:6–14, 1999.
17. Bissonette R, Zheng H, Kubo RT, Singh B, Green DR. A helper T-cell hybridoma produces an antigen-specific immunoregulatory activity: Relationship to the T-cell receptor by serology and antigenic fine specificity. J Immunol **146**:2898–2907, 1991.
18. Paliwal V, Friedman AM, Ptak W, Askenase PA. Monoclonal, antigen-specific T-cell contrasuppressor factor expresses determinants of TCR α-chain (not necessarily TCR β-chain) having a molecular mass of about 40 kDa. J Immunol **152**:2811–2820, 1994.
19. Xavier RM, Nakamura M, Tsunematsu T. Isolation and characterization of a human non-specific suppressor factor from ascitic fluid of systemic lupus erythematosus: Evidence for a human counterpart of the monoclonal non-specific suppressor factor and relationship to the T-cell receptor α-chain. J Immunol **152**:2624–2632, 1994.
20. Cone RE, Beaman KD. Antigen binding molecules of T cells (TABM) change heterogeneity and structural lability. Mol Immunol **22**:399–406, 1985.
21. Little CH, Georgiou GM, Shelton MJ, Cone RE. Production of serum immunoglobulins and T-cell antigen binding molecules specific for cow's milk antigens in adults intolerant to cow's milk. Clin Immunol Immunopathol **89**:160–170, 1998.
22. Das BK, Sahoo PK, Ravindran B. A role for tumor necrosis factor-α in acute lymphatic disease. Parasite Immunol **18**:421–424, 1996.
23. Khalil Z, Georgiou G, Ogedegbe H, Cone RE, Simpson F, Little C. Immunological and neurological studies on a benzoic acid–specific

T-cell–derived antigen-binding molecule from the serum of a patient sensitive to toluene. Arch Environ Health (in press).
24. Cone RE, Malley A. Soluble, antigen-specific T-cell proteins: T-cell based humoral immunity? Immunol Today **17:**318–322, 1996.
25. Griffith TS, Herndon JM, Lima J, Kahn M, Ferguson TA. The immune response and the eye: TCR α-chain-related molecules regulate the systemic immunity to antigen presented in the eye. Int Immunol **7:**1617–1626, 1995.
26. Wang Y, O'Rourke J, Cone RE. Serum TABM produced during anterior chamber-associated immune deviation passively transfers suppression of delayed-type hypersensitivity to primed mice. Int Immunol **9:**211–218, 1997.
27. Kelly K, Bucy RP, Nahm MH. Germinal center T cells exhibit properties of memory helper T cells. Cell Immunol **163:**206–214, 1995.
28. Casamayor-Palleja M, Kahan M, MacLennan ICM. A subset of $CD4^+$ memory T cells contains preformed CD40 ligand that is rapidly, but transiently, exposed on their surface after activation through the T-cell receptor complex. J Exp Med **18:**1293–1301, 1995.
29. Arpin C, Liu XU. Germinal center development. Immunol Rev **156:**111–126, 1997.
30. Kato K, Askenase PW. Reconstitution of an inactive antigen-specific suppressor factor by incubation of the factor with prostaglandins. J Immunol **133:**2025–2031, 1984.
31. Dimock KA, Eberbard M, Lammie PJ. Th1-like anti-filarial immune responses predominate in antigen-negative persons. Infect Immun **64:**2962–2967, 1996.
32. MacDonald AS, Maizels RM, Lawrence RA, Dransfield I, Allen JS. Requirement for *in vivo* production of IL-4, but not IL-10, in the induction of proliferative suppression by filarial parasites. J Immunol **160:**1304–1312, 1998.
33. King LL, Mahanty S, Kumaraswami V, Abrams JS, Regunathan J, Jayaraman K, Ottesen EA, Nutman TB. Cytokine control of a parasite-specific energy in human lymphatic filariasis. J Clin Invest **92:**1667–1673, 1993.
34. Olmer FM, Riley EM. Transforming growth factor β production is inversely correlated with severity of malaria infection. J Exp Med **188:**39–48, 1998.
35. Wilson ME, Young BM, Davidson BL, Mente KA, McGowan SE. The importance of TGF-β in murine visceral leishmaniasis. J Immunol **161:**6148–6155, 1998.
36. Williams M, Caspar P, Oswald I, Sharma HK, Pankewycz O, Sher A, James SV. Vaccination routes that fail to elicit protective immunity against Shistosoma mansoni induce the production of TGF-β which downregulates macrophage anti-parasitic activity. J Immunol **194:**4693–4700, 1995.