MINIREVIEW

Soluble T-Lymphocyte Antigen-Specific Immunoproteins: A Progress Report[1]

ROBERT E. CONE,[2]* GEORGE M. GEORGIOU,† AND COLIN H. LITTLE‡

Department of Pathology, University of Connecticut Health Center, Farmington, Connecticut 06030-3105; †National Aging Research Institute, Parkville 3050, Australia; and ‡Mount Waverley, Victoria 3149, Australia.

T-cell-derived proteins that bind nominal (non-MHC-associated) antigen specifically (TABM) express V and C region epitopes of the T-cell receptor (TCR) for antigen and have a significant similarity in amino acid sequence to TCR α-chain V and C region. The presence of these immunoproteins in human serum and a specific increase in serum TABM in infectious disease, chemical sensitivity, and food intolerance suggest that TABM may impact on pathogenesis through the modulation of cell-mediated immunity, the antigen-specific concentration and delivery of immunoregulatory cytokines such as TGF-β and elastase, and the induction of the release of substance P by sensory neurons. In this Minireview update, we describe advances in the detection and quantitation of human TABM by monoclonal antibodies, and the association of increased human serum TABM titers in infectious disease, chemical sensitivity, and food intolerance. We suggest that the immunomodulatory mode of action of these immunoproteins may be the antigen-specific focusing of cytokines associated with TABM.

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The specificity of the immune response is determined at the cellular level by membrane-associated antigen receptors that participate in the clonal activation of T and B cells. These specifically activated cells then effect humoral or cell-mediated activity towards the antigen. Circulating immunoglobulins can be considered the soluble expression of the B lymphocyte membrane immunoglobulin receptor for antigen because the combining site of the circulating immunoglobulin is identical to the combining site of the membrane immunoglobulin on the B cell that was the precursor of the antibody-producing plasma cell. However, the constant region that determines the effector function of secreted immunoglobulin may differ from that of the membrane immunoglobulin of the precursor of the plasma cell secreting the immunoglobulin. Moreover, the effector function of secreted immunoglobulins may be expanded by the binding of molecules (e.g., complement) produced by other cells. In contrast to B lymphocytes, much of the specific activity of T lymphocytes is cell-mediated by membrane-associated receptors (TCR) that bind antigen presented by antigen-presenting cells and associated with major histocompatibility complex (MHC) class I and class II glycoproteins. Similar to the "B cell strategy," nonantigen-specific cytokines secreted by T lymphocytes activated by antigen extend and amplify the activity of T cells to other cells. The relatively short half-life of these cytokines requires that the functional activity of these biological response modifiers is focused to an antigenic site. Thus, if cytokines were "targeted" to a specific antigenic site distant from the point of origin of the cytokine (similar to complement bound by an immunoglobulin that is binding antigen), T cell-mediated immunoregulation could be extended and effected specifically.

Although the membrane-associated TCR is specific for processed antigen presented by MHC glycoproteins, some T cells produce extracellular (soluble) "immunoproteins" that bind specifically to non-processed antigen (TABM) and mediate immunoregulation and/or hypersensitivity (reviewed in Refs. 1 and 2). TABM and functionally defined "antigen-

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[2] To whom requests for reprints should be addressed at Department of Pathology, University of Connecticut Health Center, Farmington, CT 06030–3105. E-mail: cone@idx.uchc.edu

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Table I. Soluble Antigen-Specific T Cell Proteins

Property	TsFe	TABM	TsFi
Antigen specific	+	+	+
Functions	Efferent suppression	Efferent suppression Afferent suppression	Afferent suppression
Binds nominal antigen	+	+	+
TCR epitopes	α, β	α, β	α
Unique epitope	TsFe	TsFe, TsFi	TsFi
Serum	?	+	+
Associated cytokines	Prostaglandin	TGF-β, IL-10	IL-10
Cell source	CD8	CD8, CD4, CD4–, CD8–	CD4

specific suppressor factors" share many characteristics, and TABM have themselves been shown to have immunoregulatory activity (1–6). Although TABM still need to be defined at the genomic level and further defined at the proteomic level, this updated Minireview will consider a possible mode of action and the expression of TABM in various pathologic conditions.

Properties of Soluble Antigen-Specific T-Cell Immunoproteins

Relation to the TCR. Soluble antigen-specific T-cell immunoproteins can be categorized according to their biological and/or antigen binding function (Table I). Proteins that induce regulatory T cells were termed "T suppressor-inducer factor" (TsFi, when the use of "suppressor" was academically correct), and proteins that effect the regulation of other T cells were termed "T suppressor effector factor" (TsFe). Both TsFs are M_r 50,000–110,000 and composed of an antigen-binding polypeptide chain and a biologically active, nonantigen-binding chain (1, 2, 4). TsFi and TsFe are antigenically distinct (1, 2, 4) although both share an epitope recognized by monoclonal antibodies to TCRα-chains (4, 5, 7, 8). TsFe may also possess epitopes recognized by anti-TCRβ (9). This could be due to an additional polypeptide chain; however, the expression of TsFi and TsFe depends on structural genes for TCRα, and β, respectively (7, 9), suggesting that these polypeptide chains may be derived from TCR gene products. Moreover, *in vitro* translation of mRNA for a TCRα-chain produced an M_r-30,000 polypeptide chain that binds non-processed antigen and has immunoregulatory activity (10). The amino termini of murine (T cell hybrid derived) TABM specific for azobenzenearsonate (ABA) or nitrophenyl (NP) have a similar but not identical amino-terminal sequence, and the amino acid sequence of a trytic digest of an NP-specific murine TABM shows significant similarity (but non-identity) to the C-region of murine TCRα-chains (4, 11). Human TABM also bear a TCR Cα epitope (12). Whether TABM are the products of separate, TCR-related structural genes or are a postsynthetic modification of a TCRα-chain that could result in a soluble molecule (13) remains to be determined.

Polypeptide Chain Structure. Murine TABM are >45% hydrophobic amino acids and, therefore, these hydro-

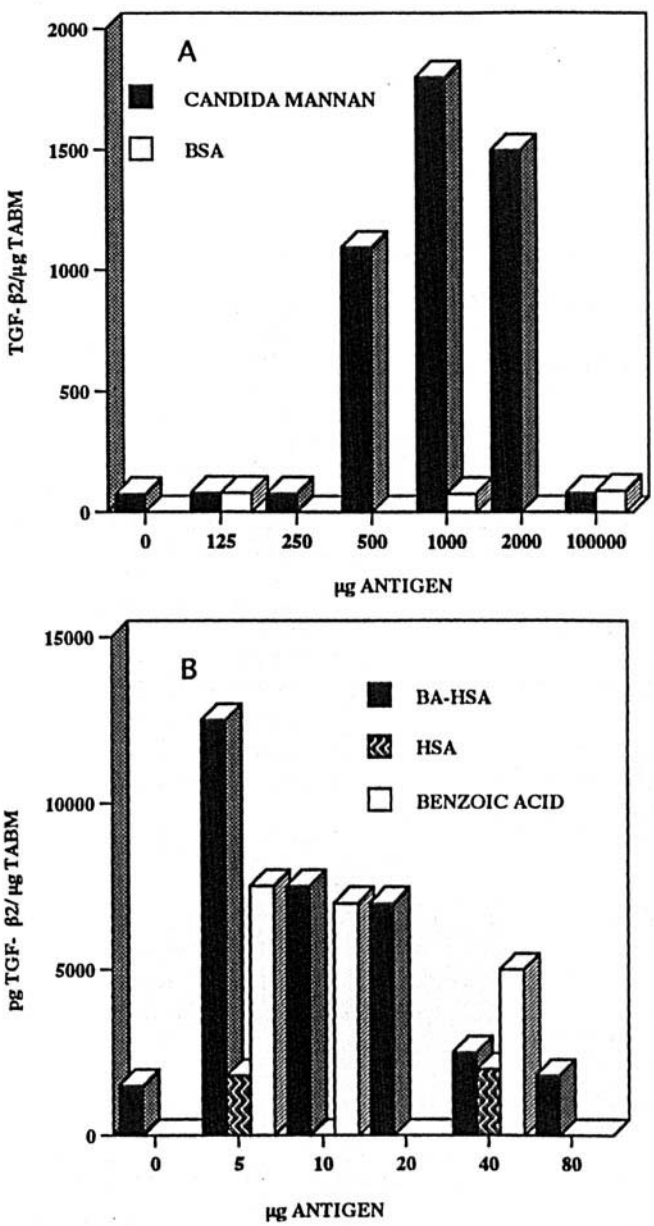

Figure 1. Specific interaction of TABM with antigen increases (antigenically) active associated TGF-β. Five hundred nanograms of serum TABM isolated by monoclonal antibody (3C9) and affinity for *Candida* mannan (A) or benzoic acid (B) were incubated with the designated antigens for 30 min and were then assayed for TGF-β2 by ELISA.

phobic molecules are difficult to study (11). Although TABM bear TCR Cα and sometimes Cβ epitopes and their expression depends on TCR structural genes, (limited) amino acid sequence suggests that TABM are not TCR chains (11). Many sizes ranging from M_r 22,000 to 33,000 protomers to M_r 110,000 or to aggregates $>10^6$ have been reported (1–4). The polydisperse nature of TABM may be due to their hydrophobic properties that facilitate aggregation. TABM found in serum (6, 12, 14) are precipitated by 43%–50% ammonium sulfate, have the electrophoretic mobility of α globulins (14), and have a molecular size $>10^6$ (12) as evidenced by molecular sieving of non-denatured molecules. These properties of TABM indicate that TABM may "contaminate" preparations of other serum proteins. Reduction and denaturation of these large multimers produces M_r-33,000 molecular species. In addition, TABM may often be non-covalently associated with cytokines (*vide infra)* and, therefore, may be perceived as large structures composed of different polypeptide chains.

Serum TABM Response to Immunization. Immunization of mice with protein antigens (15) or trinitrophenylated cells (5, 16) induces an increase in serum of TABM specific for the immunogen when the immunogen is injected with an adjuvant. In these studies, antigen-specific TABM were detected in immune serum by a sandwich ELISA that used antigen-coated microtiter trays and polyclonal or monoclonal anti-TABM to detect serum TABM binding to antigen (2, 5, 15–17). Titers of serum TABM rise when immunization favors cell-mediated immunity, although the rise is more pronounced during a humoral immune response (2, 5, 15, 16). SCID mice do not produce TABM after immunization. However, TABM but not immunoglobulins are produced by SCID mice reconstituted with thymocytes (15), suggesting that serum TABM are produced by T cells but not B cells. In addition, TABM specific for the immunogen may be detected when immunoglobulins specific for the immunogen are not produced (12, 16). In fact, some TABM specific for the same epitope

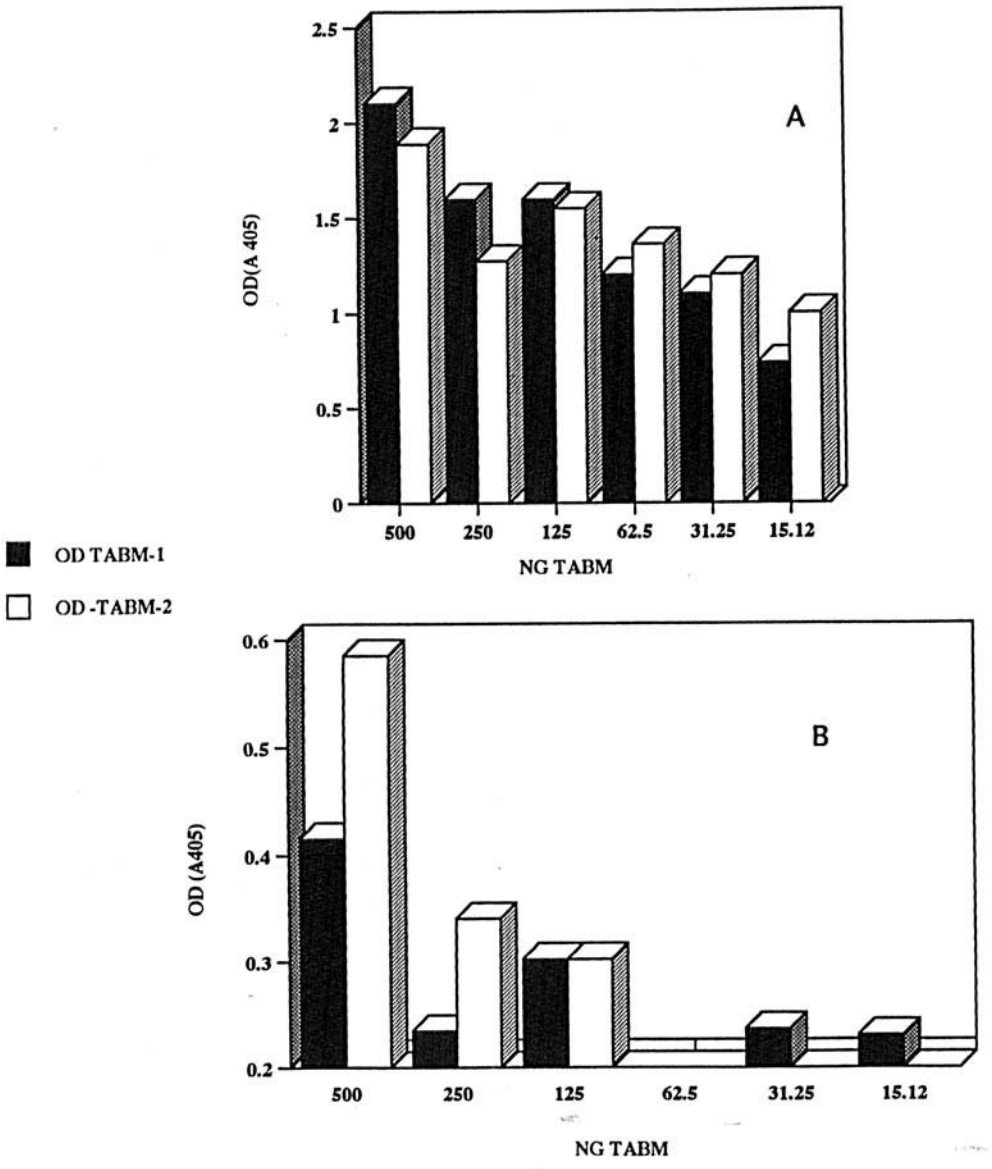

Figure 2. Proteolytic activity and elastase associated with TABM. Benzoic acid-specific TABM isolated by monoclonal 3C9 and affinity for benzoic acid from the sera of toluene-sensitive patients were coated to microtiter trays and assayed by ELISA with monoclonal anti-TABM 3C9 (A) or monoclonal anti-human elastase (B).

as immunoglobulins are detected more readily when immunoglobulins are removed from the immune serum before the antigen-capture ELISA (Malley A, personal communication). These observations suggest that immunoglobulins may compete with TABM for the same epitope because the serum concentration of TABM is <50 μg/ml (14).

Although TABM express a TCRα epitope, unlike the TCR, TABM bind specifically to nominal antigen. However, TCRα+,β- TABM bind nominal antigen, but TCRα+β TABM do not bind nominal antigen (6, 12, 16). Perhaps an associated polypeptide chain *prevents* the binding of nominal antigen. Moreover, TABM are highly aggregated (>10 protomeric units), and these multimers may be able to ligate nominal antigen. In this regard, the avidity of TABM for antigen is 10- to 100-fold less than that of immunoglobulins, further complicating the detection of TABM in an immune serum. Despite this, ELISA of immune serum for TABM demonstrated that the rise in antigen-specific TABM during immunization peaks 24–48 hr earlier than the peak of immunoglobulins, and TABM titers decrease, whereas immunoglobulin titers are maintained (15). This decrease in serum TABM specific for the immunogen could be due to a cessation of TABM production and/or competition between TABM and immunoglobulins for the same epitope. A secondary challenge induces a more rapid increase in TABM for the immunogen and a higher TABM titer, indicating that TABM production exhibits immunologic memory (15). Because TABM are a T cell product, the serum TABM response reflects a *humoral* T cell response. Human TABM are produced by CD4 or CD8 T cells (3) but not B cells (12). As discussed below, the cellular origin of the TABM may influence their function.

Serum TABM Regulate Delayed-Type Hypersensitivity (DTH). The injection of antigen into the anterior chamber (AC) of an eye of a mouse or a rat and immunization of the animal to the same antigen inhibits cell-mediated immunity and IgG2 antibody production to the antigen. The production of IgM and IgG1 antibody to the antigen is not affected or may even be enhanced. This anterior chamber-associated immune deviation (ACAID) has been a powerful tool in dissecting the activation of regulatory T lymphocytes and eye-immune system interactions (reviewed in Ref. 18). TCR $C\alpha^+$ TsFi has been demonstrated in the sera of mice receiving an AC injection of herpes virus (8). AC injection of naive mice with trinitrophenylated (TNP) spleen cells (17), proteins, or trinitrophenylated proteins (5, 19) potentiated or induced the appearance of TABM specific for the AC-injected antigen. That these serum TABM mediate the suppression of DTH imposed by intracameral injection of antigen is demonstrated by several results: Injection of the mice with cyclophosphamide prevented ACAID and the production of TABM (20); ACAID (21) or serum TABM (Wang Y, Cone RE, unpublished observations) cannot be induced in mice thymectomized as adults; and, TCR $C\alpha^+$ antigen-specific serum TABM produced by mice with ACAID suppress the induction of DTH when injected into immunized mice (5).

TABM Are Associated with Cytokines. The mechanism(s) by which TABM suppress DTH has not been elucidated. However, TNP-specific TsFe that effects the suppression of DTH was shown to be associated with prostaglandin (22). Moreover, TsF prepared by incubation of TABM-producing cells *in vitro* with indomethacin was not suppressive providing more evidence, suggesting that prostaglandin associated with TsF suppressed DTH. NP-specific TABM produced *in vitro* by a T cell hybrid are noncovalently associated with IL-10 produced by the T cell hybrid (4). In addition, mice with ACAID to TNP produce TNP-specific serum TABM associated with IL-10 (Wang Y, Cone RE, unpublished observations). These IL-10-associated TABM will suppress DTH to TNP when injected i.v. into TNP-sensitized mice. Although TABM are not produced by adult-thymectomized mice, injection of TNP-specific, ACAID-inducing TABM do not induce ACAID when injected into adult-thymectomized-mice (Wang Y, Cone RE, unpublished observations). These findings suggest that the induction of ACAID by TABM may require the *presence* of thymic regulatory T cells activated by TABM and antigen.

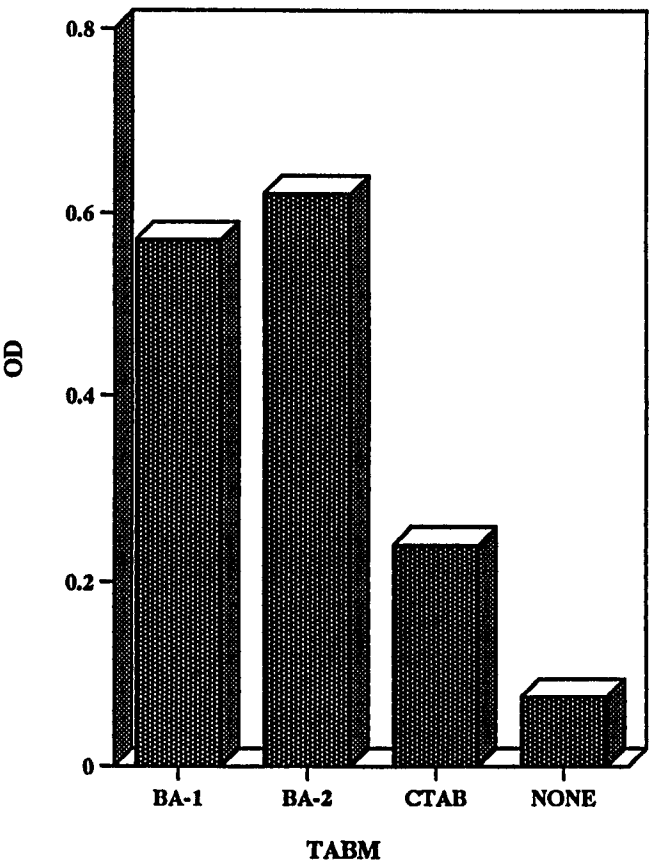

Figure 3. Proteolytic activity associated with TABM. Benzoic acid-specific TABM (Fig. 2) or TABM specific for *Candida* mannan (CTAB, Fig. 1A) were assayed for proteolytic activity using the Pierce Protease Assay. The increase in the optical density of dinitrophenol (cleaved from DNP-albumin) is an indicator of the proteolysis of DNP-BSA.

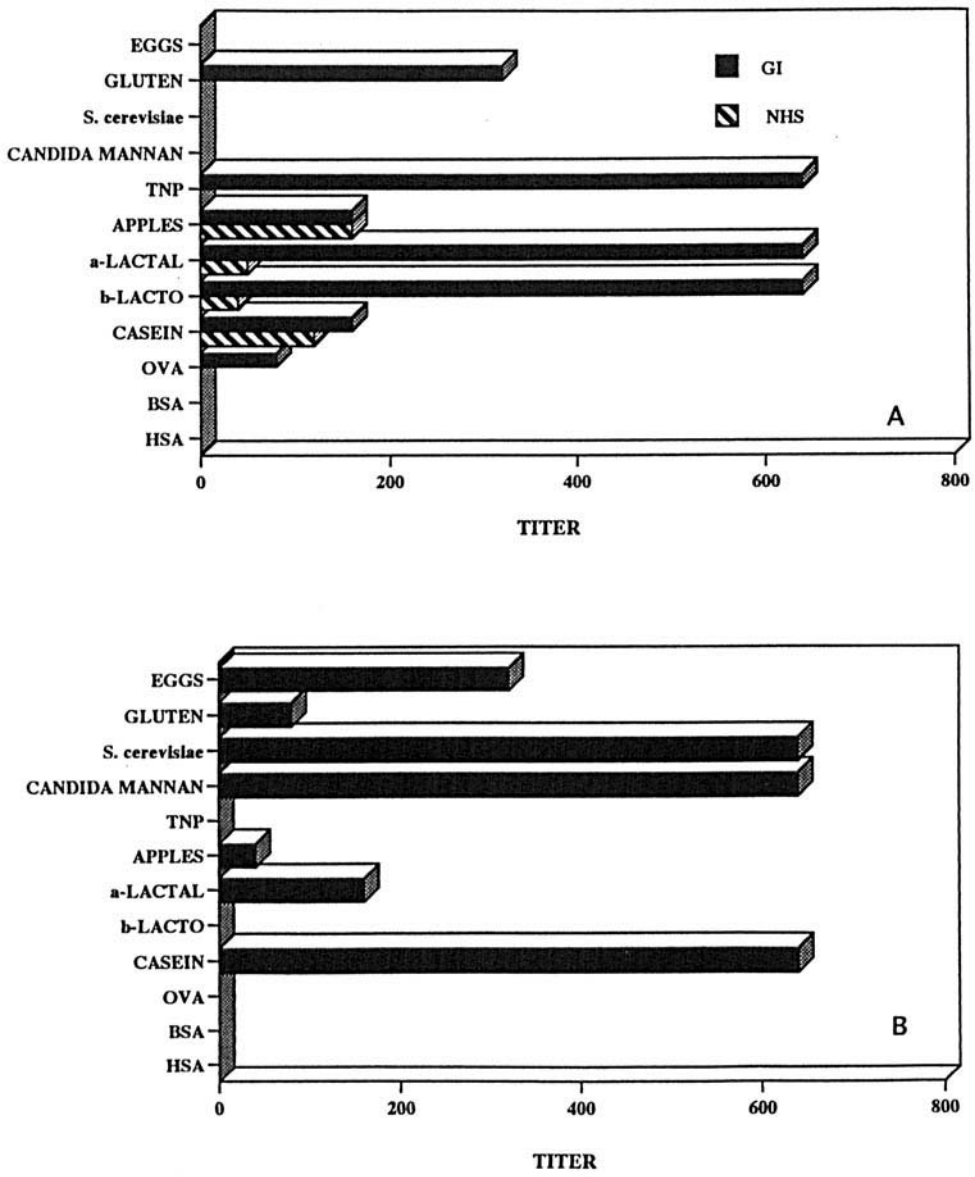

Figure 4. Antigen binding by serum TABM in gastrointestinal disorder. Serial dilutions of sera from two patients (A and B) with gastrointestinal disorder or serum from a non-affected individual (NHS) were added to microtiter trays coated with the designated antigens. Bound TABM were detected by the addition of monoclonal anti-TABM 3C9 and alkaline phosphatase-conjugated anti-murine IgG. The titer is the reciprocal of the highest dilution of the serum giving an optical density 2-fold higher than that obtained without the addition of the serum. a-Lactal, α-lactalbumin; b-Lacto, β-lactoglobulin; BSA, bovine serum albumin; OVA, ovalbumin; HSA, human serum albumin; TNP, trinitrophenylated BSA.

Human TABM non-covalently associated with TGFβ1 or TGFβ2 are found in non-immune (12) sera and antigen-specific TABM from the sera of individuals sensitized to toluene (benzoic acid specific) (6, 23), milk proteins (casein, α-lactalbumin, and β-lactoglobulin) (24), or *Candida* (mannan) (6). As shown in Figure 1 (A and B), the amount of TABM-associated TGF-β detected by ELISA increases or decreases specifically when the TABM are incubated with homologous antigen (6, 23). The dose-response relationship showing the increase in TGFβ at optimal concentrations of specific antigens suggests that the interaction of the TABM with antigen "activates" the associated TGFβ. The activated TGFβ would suppress the production of IFN-γ by T lymphocytes (6). TCRCα$^+$ non-specific suppressor factor detected in human ascitic fluid is associated with TGFβ (25). Perhaps the non-specific suppressor factor may be TABM associated with TGFβ.

TABM may function by antigen-specific "targeting" of cytokines. Although to date only prostaglandin, IL-10, or TGFβ have been detected associated with TABM, other cytokines produced by a TABM-producing cells may also be associated with TABM. It is probable that TABM are associated with more than one cytokine. In addition, elastase has been found to be functionally and physically associated with a murine antigen-specific suppressor factor produced by a T cell hybrid (26). Our laboratory has detected elastase associated with a benzoic acid-specific TABM (Fig. 2) isolated by monoclonal anti-TABM and affinity for benzoic acid. Perhaps proteolytic activity associated with this TABM (Fig. 3) is responsible for proteolytic "activation" of TGF-β after the TABM binds antigen. Moreover, the focusing of cytokines by TABM, particularly TGFβ, could lead to untoward collateral effects in addition to immunoregulation. TGFβ associated with TABM will induce the secretion of substance P and will amplify the effect of substance P on sensory neurons (23). As discussed below,

Table II. Elevated Serum TABM

Disorder	Antigen
Vulvovaginal candidiasis	*Candida* mannan *S. cerevisiae*
Filariasis	Filarial extract
Toluene sensitivity	Benzoic acid
Milk intolerance	Casein, β-lactoglobin, and α-lactalbumin
Melanoma	Mage III peptide antigen
AIDS (symptomatic)	Non-specific increase and HIV surrogate peptide
Inflammatory Bowel Syndrome	Casein, α-lactalbumin, benzoic acid, gluten, ovalbumin, eggs, and *Candida* mannan, *S. cerevisiae*
Latex sensitivity	Avocado, walnut, and eggplant

TABM could increase pain, muscle contractions, and mast cell-mediated effects via the action of TABM-derived TGFβ on the release and activity of substance P.

Monoclonal Anti-(Human) TABM. The characterization and definition of TABM has been complicated by the hydrophobic nature of these polypeptides, the use of only polyclonal antibodies to human TABM, and the lack of cloning of TABM structural genes. These problems have been approached by the generation and use of monoclonal antibodies to human TABM (12). Human serum Cohn Fraction III proteins rich in TABM were fractionated further by ammonium salt precipitation, cationic exchange chromatography, and immunoadsorption of the fractionated proteins. The resulting proteins were $>M_r$ 10^6 polypeptides that, when reduced and denatured, were resolved by SDS-PAGE as M_r 33,000 proteins. These proteins reacted with polyclonal anti-TABM antibodies in ELISA and monoclonal antibodies to human TCR Cα-chains, but were not recognized by antibodies to human immunoglobulin μ,γ,α-chains, immunoglobulin κ,λ-chains, or human serum albumin. However, TGFβ (12) and elastase (Fig. 2) were detected in the TABM preparation. Monoclonal antibody prepared against these proteins recognized $TABM^+$ preparations, M_r 33,000 serum proteins, and proteins produced by HUT78 (T cell) lymphoma in culture, but did not bind to human serum albumin, TGFβ, B lymphoma proteins immunoglobulins (12), or elastase (Cone RE, unpublished observations). Monoclonal anti-TABM does not detect lymphocyte surface proteins, but does detect intracellular proteins on some mononuclear cells (12). The identity of these cells has not been made, but $TABM^+$ cells have been detected in the T cell zone of germinal centers (12).

Detection of TABM to Infectious Agents, Food Antigens, or Chemicals in Infectious Disease, Gastrointestinal Disturbance, or Chemical Sensitivity. Serum TABM specific for various antigens have been detected in human sera by ELISA with polyclonal (14, 23) or monoclonal (6, 12, 23) anti-TABM. As shown in Figure 4, sera from individuals with suspected inflammatory bowel disease have elevated TABM titers to several food antigens. Elevated serum TABM titers to casein or α-lactalbumin have been found in individuals intolerant to milk (12, 24) (Table II). Women susceptible to recurrent vulvovaginal candidiasis have elevated serum TABM titers to Candida mannan (6), which may indicate a basis for reduced cell-mediated immunity to Candida in such individuals. Similarly, individuals with chronic or asymptomatic filariasis have elevated serum TABM titers to filarial antigens (12), which may reflect reduced cell-mediated immunity to filaria. Patients sensitized to toluene (by industrial exposure) and symptomatic to an exposure to toluene or benzene have elevated serum TABM titers specific for benzoic acid (a breakdown product of toluene) (23). Antigenically (biologically) active TGFβ associated with benzoic acid-specific or Candida mannan-specific TABM specifically increased when these TABM were incubated with antigen in optimal proportion (6, 23) (Fig. 1). For benzoic acid-specific TABM, this effect was observed for both conjugated and unconjugated benzoic acid. The activated TGFβ may induce neurological effects directly or through substance P (23). Accordingly, we suggest that the antigen-specific con-

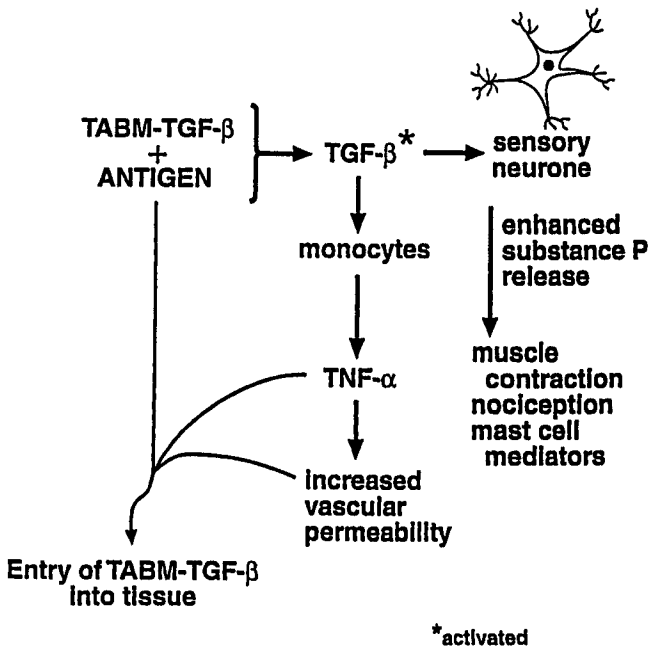

Figure 5. A model for the affect of TABM: antigen interaction on sensory neurons through TABM-associated TGF-β. TABM-TGF-β produced by T cells bind specifically to antigen and TABM-associated TGF-β is thereby activated. The activated TGF-β increases the production of substance P by sensory neurons and induces the production of TNF-α by monocytes that increases the entry of TABM into tissue by increasing vascular permeability.

centration of cytokines, particularly TGFβ, may produce untoward effects (Fig. 5). In addition, the apparently nonspecific elevation of serum TABM in HIV$^+$ symptomatic individuals (3) may be responsible for an early downregulation of cell-mediated immunity to the virus. Thus, the detection of antigen-specific TABM indicates that these immunoproteins represent a relatively uncommon immune response that, in fact, may be necessary to achieve a protective balance against more damaging cell-mediated immunity. However, like any other "double-edged sword" of the immune system, TABM themselves may participate in some pathologic conditions. An increase in a TABM titer specific for melanoma antigen MAGE III peptide have been detected in the sera of some individuals with melanoma (3) and in individuals vaccinated with MAGE III-peptide-pulsed antigen-presenting cells that did not produce T cells cytotoxic for melanoma cells (3). Thus, in addition to mediating certain pathologic conditions, TABM may provide an indicator of the efficiency of vaccination to induce humoral and/or cell-mediated immunity.

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