

# Human Immune Response against Timothy Grass Pollen Allergens

## I. Shared Idiotypes on Human and Murine Antigen-B-Specific Lymphocyte Receptors<sup>1</sup>

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**Abstract.** Enzyme-linked immunoassays were used to examine the binding specificity of affinity-purified anti-idiotypic antibody [against the idiotypic determinant on timothy grass pollen antigen B (AgB)-specific IgE] with murine AgB-specific IgG and IgE antibodies, and the serum from timothy-sensitive patients. In addition, the proliferative response of peripheral blood lymphocytes from timothy-sensitive patients with either antigen or anti-idiotypic antibody was examined. These studies suggest that the idiotypic determinant expressed on murine AgB-specific IgE is highly conserved on human AgB-specific IgE and T cells of timothy-sensitive patients.

### Introduction

Idiotypic analysis of immunoglobulins has provided a means of probing antibody responses, diversity and regulation [1]. Much of the information has been obtained through the use of antigen systems that induce a restricted response involving certain dominant idiotypes [2-7]. The responses of several mouse strains to immunization with an aqueous extract of timothy grass pollen extract (WST) adsorbed on alum appear to be restricted primarily to the antigenic determinants on antigen B (AgB) [8, 9]. Previously, we described the isolation of AgB-specific IgE and its use to prepare anti-idiotypic antibody (anti-Id) [10].

Characterization of the AgB-specific anti-Id demonstrated the following: (1) the anti-Id induces significant <sup>3</sup>H-thymidine incorporation of AgB-specific helper and suppressor T cells in the absence of antigen; (2) preincubation of AgB-specific B cells with anti-Id leads to a 6- to 8-fold enhancement of AgB-specific IgE formation, and (3) AgB-specific T helper factor and T suppressor factor share the same idiotypic determinant expressed by AgB-specific IgE [10].

Conservation of idiotypic determinants across species has been reported to a variety of antigens [11-13]. Recently, shared idiotypic determinants on both human IgG and IgE antibodies directed against major allergens of house dust and ryegrass pollen have been reported [14, 15]. In the study reported here, we attempt to determine if the idiotypic determinants expressed on murine AgB-specific T and B cells is also expressed on human T cells and IgE made by patients allergic to timothy grass pollen.

### Materials and Methods

#### *Reagents and Buffers*

Sephacrose 4B was obtained from Pharmacia (Piscataway, N.J., USA); CNBr from Pierce Chem. (Rockford, Ill., USA); urea from Baker (Phillipsburg, N.J., USA); fetal calf serum (FCS) from Flow Laboratories (Inglewood, Calif., USA); normal mouse serum (NMS) from Miles Scientific (Naperville, Ill., USA); glycine-buffered saline (GBS) 0.2 M, pH 3, and phosphate-buffered saline (PBS), pH 7.4, were used.

#### *Antigens*

Water-soluble timothy pollen extract (WST), AgB, antigen D (AgD) and photo-oxidized AgB (OX-AgB) were prepared as previously described [16, 17]. The ragweed pollen extract was a gift from Dr. George Fukui (Wallace Laboratories, Princeton, N.J., USA). The cellobiose was purchased from Calbiochem (San Diego, Calif., USA). The beta-gentiobiose, alpha-maltose, alpha-isomaltose and

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salicin were purchased from Aldrich Chemical (Milwaukee, Wisc., USA).

#### *Antisera*

MOPC104E (murine IgM) and a murine monoclonal antihuman IgE were obtained from Miles-Yeda (Rehovot, Israel). Monoclonal AgB-specific IgG antibodies were obtained following fusion of enriched B cells of mice primed with OX-AgB absorbed on 1 mg of alum with P3X63Ag8.653 B cells. The resulting hybrid cells were cloned twice by limiting dilution, and the cell-free supernatants of these cultures were purified by passage over Sepharose-protein A. The monoclonal AgB-specific IgG1 antibody was eluted with GBS, dialyzed against PBS and concentrated by negative pressure. Trinitrophenyl (TNP)-specific IgE monoclonals (TIB141, TIB142) were obtained from the American Type Culture Collection (ATCC). AgB-specific IgE was obtained from hyperimmune mice. Sera from these animals were precipitated by ammonium sulfate (35% saturated), and the supernatant material was dialyzed to remove ammonium sulfate. The murine TNP-specific IgE and AgB-specific IgE were further purified by DEAE-high-performance chromatography (HPLC) using a 20-mM Tris buffer, pH 8.0, and a linear gradient of 20 mM Tris buffer, pH 7.0. The murine AgB-specific IgE was further purified by passage over a Sepharose-AgD adsorbent, and the concentrated eluate from this adsorbent had a passive cutaneous anaphylaxis (PCA) titer of 1:12,000. The purity of AgB-specific IgE was determined by SDS-PAGE [19, 20]. Serum samples from timothy- and ragweed-sensitive patients were provided by Drs. John O'Holloren (Portland, Oreg., USA) and Charles Reed (Minneapolis, Minn., USA). Anti-Lyt-1 antibody was prepared from a hybridoma cell line (53-7.313; ATTC), and the IgG2a purified by HPLC.

#### *Preparation of Anti-Id*

Rabbit anti-Id was prepared by subcutaneous injection of AgB-specific IgE mixed with an excess of rabbit anti-NMS. The isolation and characterization of the anti-idiotypic properties of the rabbit anti-AgB-specific IgE has been described elsewhere [10]. The anti-Id was adsorbed by passage over Sepharose-mouse IgG, mouse light chains, FCS, NMS adsorbents, anti-TNP-specific IgE, and purified by passage over a Sepharose-anti-AgB-specific IgE adsorbent. The bound anti-Id was eluted from the Sepharose-anti-AgB-specific IgE adsorbent with GBS, dialyzed against PBS and concentrated by negative pressure. The affinity-purified anti-Id was used in all the experiments discussed. The affinity-purified anti-Id did not show any significant binding with 1 µg of IgG, kappa or lambda light chains, or 2 TNP-specific IgE monoclonal antibodies in an enzyme-linked immunosorbent assay (ELISA). In addition, the anti-Id does not block the interaction of OX-AgB and monoclonal anti-AgB IgG with either polyclonal anti-AgB IgG or any of the 17 antibodies examined.

#### *Immunoabsorbents*

Sepharose-protein A was purchased from Pharmacia and immobilized cellobiose from Pierce. The preparation of Sepharose-AgD, Sepharose-anti-AgB-specific IgE and the conjugation of other patients to Sepharose 4B have been described elsewhere [10].

#### *ELISA*

Antigen-specific human IgE was measured by coating microtiter ELISA plates with 200 µl of WST (1 mg/ml) or ragweed pollen

extract (0.5 mg/ml) in coating buffer for 3 h at 37°C. The plates were washed 3 times with washing buffer, and patients' serum or normal human serum (diluted 1:50) was incubated overnight at room temperature in a moist chamber. The plates were washed and incubated with mouse monoclonal antihuman IgE (diluted 1:8,000) overnight at room temperature in a moist chamber. The plates were washed and incubated with rabbit antimouse IgG-alkaline phosphatase (1:200) for 3 h at 37°C. The plates were washed 3 times with washing buffer and 3 more times with substrate buffer followed by the addition of substrate for 30 min at 37°C. The reaction was stopped by the addition of 50 µl of 3 M NaOH, and the developed color was measured in an ELISA reader (Bio-Tek, Burlington, Vt., USA). In experiments designed to determine if the idiotype present on murine antitimothy IgE was also present on human IgE directed against AgB, 20 µg of rabbit antitimothy IgE (anti-Id) was incubated for 1-3 h at room temperature with the diluted serum samples from ragweed- (Prausnitz-Keustner titers 1:500 for ragweed and negative to timothy pollen) and timothy-sensitive (Prausnitz-Keustner titers 1:500-1:5,000 for timothy pollen extract) patients prior to the addition of these mixtures to antigen-coated wells. If the shared idiotype determinants exist on human and murine AgB-specific IgE, the addition of anti-Id will decrease the level of timothy-specific IgE bound in the ELISA described above.

Characterization of the binding site associated with the idiotype determinant expressed on AgB-specific IgE was achieved by coating microtiter ELISA wells with 100 µl of the affinity-purified AgB-specific IgE (1:1,000 PCA titer). Plates were washed with washing buffer and incubated with varying concentrations of diglucoside molecules or salicin for 1 h at room temperature prior to the addition of 5 µg of anti-Id. The plates were incubated overnight at room temperature in a moist chamber. After being washed, the plates were incubated with sheep antirabbit IgG-alkaline phosphatase (1:1,500) for 3 h at 37°C followed by the addition of substrate for 60 min at 37°C. OX-AgB, which does not bind AgB-specific IgE, was used to measure the binding of monoclonal AgB-specific IgG. Microtiter wells were coated with 50 ng of OX-AgB, washed 3 times and incubated for 2 h at 37°C with 50 ng of monoclonal AgB-specific IgG (D7.4.10) antibody. The level of IgG bound was determined by the addition of rabbit antimouse IgG (γ-chain-specific)-alkaline phosphatase and substrate. Experiments to determine the cross-reactivity of idiotype determinants expressed on murine AgB-specific IgG and IgE involved the incubation of 20-100 µg of anti-Id (rabbit antimouse AgB-specific IgE) to monoclonal anti-AgB IgG prior to the addition of this mixture to the OX-AgB-coated wells.

#### *Antigen-Induced Proliferation*

Human peripheral blood lymphocytes from timothy-grass-sensitive patients (Prausnitz-Keustner titers 1:500-1:5,000 for timothy pollen), nonallergic individuals and nongrass-sensitive patients were separated by Hypaque-Ficoll gradients and cultured with either varying concentrations of AgB (1-10 µg) or anti-Id (0.3-3.6 µg) by methods previously described [18]. The lymphocytes ( $2 \times 10^5$ ) were cultured for 5 days at 37°C in 5% CO<sub>2</sub> and pulsed with 1 µCi of <sup>3</sup>H-thymidine during the last 18 h of culture. The cells were harvested by a minimultiple automatic sample harvester (Microbiological Assoc., Los Angeles, Calif., USA) and counted by liquid scintillation methods.

## Results

### *Cellobiose Inhibition of AgB-Specific IgE-Anti-Id Interaction*

Previous structural analysis of AgD, a monovalent antigen fragment of AgB, demonstrated that cellobiose represents a major part of the antigenic determinant of AgB [21]. Consequently, we used cellobiose, and varying concentrations of a variety of other disaccharides and salicin, to block the binding of anti-Id with AgB-specific IgE (fig. 1). Increasing concentrations of cellobiose (up to 12  $\mu$ M) resulted in a linear dose-dependent inhibition of idiotype-anti-Id interaction. In contrast, the addition of stereoisomers of other diglucoside molecules and salicin (up to 200  $\mu$ M) did not result in any inhibition of the idiotype-anti-Id interaction. Cellobiose (up to 200  $\mu$ M) does not affect ovalbumin-antiovalbumin, bovine serum albumin (BSA)-antiBSA or murine IgM (MPOC104E)-anti-IgM interaction (data not shown).

### *Failure of Anti-Id to Inhibit OX-AgB Interaction with Monoclonal Anti-AgB IgG Antibody*

Previous studies have demonstrated that OX-AgB neither induces nor reacts with AgB-specific IgE [17]. However, OX-AgB induces a significant AgB-specific IgG response in a number of mouse strains [9]. Figure 2 shows the binding of varying amounts of monoclonal anti-AgB IgG antibody with OX-AgB. The addition of increasing amounts of antibody results in a linear dose-dependent increase in binding. The incubation of 20–100  $\mu$ g of anti-Id or 200  $\mu$ M of cellobiose with monoclonal anti-AgB IgG antibody for 2 h at room temperature prior to the addition of these mixtures to OX-AgB-coated wells did not block the binding of the anti-AgB IgG antibody with OX-AgB. Although the data for the interaction of 1 monoclonal anti-AgB IgG antibody (clone D7.4.10) are presented, 17 different monoclonal anti-AgB IgG antibodies have been examined. The interaction of OX-AgB and these monoclonal anti-AgB IgG antibodies was not blocked by the addition of rabbit anti-Id.

### *Anti-Id Inhibition of Human Timothy-Specific IgE Binding with WST*

The apparent dominance of the idiotypic determinant expressed on murine T and B lymphocytes directed against AgB seems to parallel the dominance found in earlier studies that demonstrated the successful inhibition by AgD of significant levels of antigen-



**Fig. 1.** Inhibition of idiotype-anti-idiotype interaction by cellobiose. Microtiter wells were coated with 100  $\mu$ l of AgB-specific IgE (PCA titer of 1:1,000) suppressor factor for 3 h at 37°C. Nonspecific binding sites were blocked by the incubation of BSA (1 mg/ml) in coating buffer for 1 h at room temperature. The wells were washed and various concentrations of disaccharides were added for 1–2 h at room temperature prior to the addition of 5  $\mu$ g of anti-Id. The plates were incubated at room temperature overnight, and the bound anti-Id detected by the addition of antirabbit IgG-alkaline phosphatase and substrate.

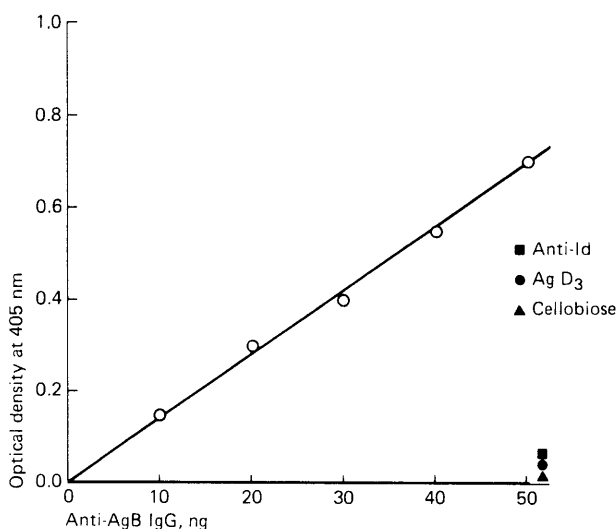


**Fig. 2.** Failure of anti-Id, cellobiose or AgD<sub>3</sub> to block the binding of monoclonal anti-AgB IgG (clone D7.4.10) antibody with OX-AgB. 50 ng of OX-AgB in coating buffer, pH 9.6, was coated on microtiter wells overnight at 4°C. Unreacted sites were blocked with BSA (1 mg/ml) in coating buffer. Wells were washed with PBS-Tween 20 and incubated with varying amounts of anti-AgB IgG antibody for 3 h at 37°C. The bound antibody was detected by the addition of rabbit antimouse IgG ( $\gamma$ -chain-specific)-alkaline phosphatase and substrate. In experiments attempting to block anti-AgB IgG antibody binding, 200  $\mu$ M of cellobiose, 10  $\mu$ g of AgD<sub>3</sub> or up to 100  $\mu$ g of anti-Id was incubated with the anti-AgB IgG antibody for 2 h at room temperature prior to the addition of these mixtures to OX-AgB-coated wells.

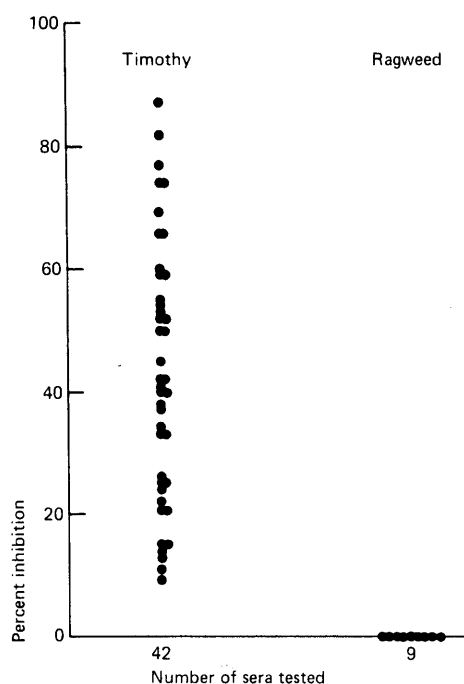


**Fig. 3.** Anti-Id inhibition of human AgB-specific IgE binding to WST-coated microtiter wells. Microtiter wells were coated with 200  $\mu$ g of protein of WST for 3 h at 37°C, washed 3 times with PBS-Tween 20; 1:50 dilutions of human serum from timothy-sensitive patients were incubated with either 30  $\mu$ g of affinity-purified normal IgG or 30  $\mu$ g of affinity-purified anti-Id for 1–2 h at room temperature prior to the addition of these mixtures to the antigen-coated wells. The plates were incubated overnight at room temperature, washed and the levels of human antithimothy IgE were measured by the addition of monoclonal antihuman IgE. Control antisera from ragweed-pollen-sensitive patients were added to ragweed-extract-coated wells after incubation with either affinity-purified normal IgG or anti-Id. The detection system used antimouse IgG-alkaline phosphatase and substrate.

induced histamine release from monkey mast cells [21, 22]. Those studies showed that monkey mast cells passively sensitized with serum from timothy-sensitive patients show a linear, dose-dependent inhibition of antigen-induced histamine release upon the addition of AgD prior to the WST challenge. These earlier observations and our more recent findings raise a

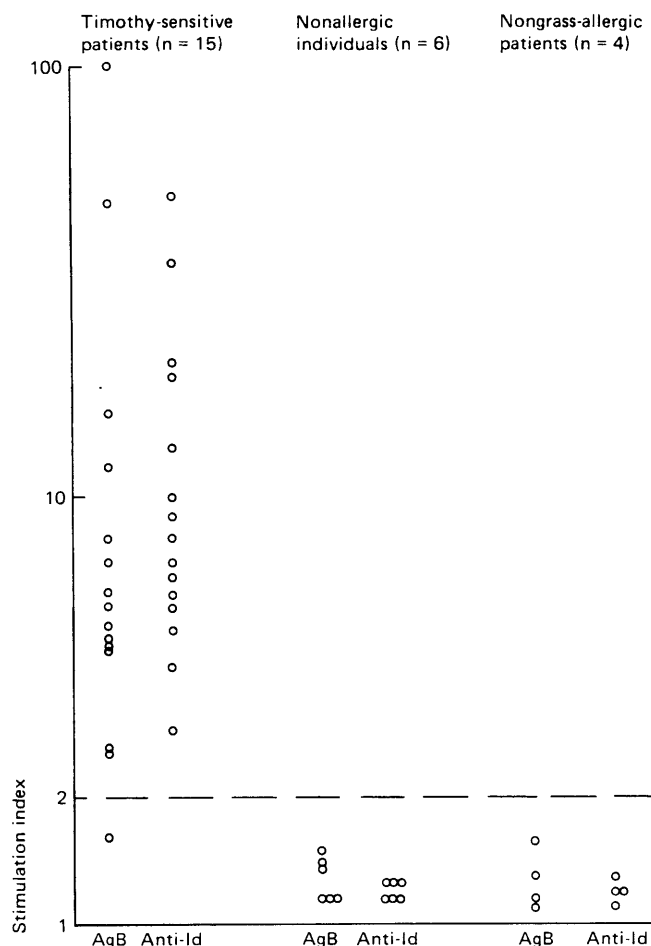


**Fig. 4.** AgB- and anti-Id-induced proliferation of human peripheral blood lymphocytes. Human peripheral blood lymphocytes from timothy-grass-sensitive patients, nonallergic individuals and nongrass-sensitive patients were separated by Hypaque-Ficoll gradients and cultured with either varying concentration of AgB or anti-Id. The lymphocytes ( $2 \times 10^5$ ) were cultured for 5 days at 37°C in 5% CO<sub>2</sub> and pulsed with 1  $\mu$ Ci of <sup>3</sup>H-thymidine during the last 18 h of culture. The cells were harvested and counted by liquid scintillation methods. The stimulation index was calculated as:

$$\frac{\text{experimental cpm} - \text{background cpm}}{\text{background cpm}}$$

question regarding the possible conservation of the idiotypic determinant expressed by murine AgB-specific lymphocyte receptors and the idiotypic determinant expressed by human AgB-specific IgE. Serum from timothy-sensitive patients preincubated with 20  $\mu$ g of affinity-purified anti-Id prior to the addition of this mixture to microtiter wells coated with WST

showed significantly blocked IgE binding. Of the serum samples tested, 70% (29/42) showed between 35 and 87% inhibition; the remaining samples showed inhibition between 10 and 30% (fig. 3). Preincubation of the serum from timothy-sensitive patients with affinity-purified rabbit IgG prior to the addition of this mixture to WST-coated wells gave IgE-binding levels that were indistinguishable from serum samples mixed with incubation buffer. Serum samples from ragweed-sensitive patients preincubated with up to 100  $\mu$ g of anti-Id prior to the addition of this mixture to ragweed-coated microtiter wells were not inhibited (fig. 3).

#### *Specificity of AgB and Anti-Id-Induced Human Lymphocyte Proliferation Responses*

Previously, we have shown that anti-Id induces a dose-dependent incorporation of  $^3$ H-thymidine into murine AgB-specific helper and suppressor T cells cultured with anti-Id [10]. The inhibition by AgD of AgB or WST-induced proliferation of human peripheral blood lymphocytes of timothy-sensitive patients suggested that this monovalent fragment interacts with the antigen-binding site of human AgB-specific T cells [18, 23]. Therefore, we examined the ability of anti-Id to initiate proliferation of human peripheral blood lymphocytes from timothy-sensitive patients, nonallergic individuals or patients allergic to a variety of noncross-reactive allergens (fig. 4). Patients allergic to timothy grass pollen gave a dose-dependent increase in the incorporation of  $^3$ H-thymidine when stimulated with either AgB or anti-Id (data not shown). Stimulation indices of lymphocytes from timothy-sensitive patients cultured with AgB varied from 3.2 to 100, and varied from 4.1 to 50 with anti-Id. In contrast, peripheral blood lymphocytes of nonallergic individuals or patients allergic to noncross-reacting allergens cultured with either AgB or anti-Id did not induce proliferative responses above control cultures (cells only).

#### **Discussion**

The stereospecific inhibition by cellobiose of idiotype-anti-idiotypic interaction (fig. 1) not only confirms earlier data indicating its participation in the antigenic determinant of AgB [21, 22], but also suggests that cellobiose is directly involved in the binding site of the antigenic determinant. Reth et al. [7] and

Kranz and Voss [24], analyzing various monoclonal anti-Id, have suggested that inhibition of idiotype-anti-Id interaction by free hapten represents direct binding of the hapten with the idiotope and that inhibition with a hapten-protein conjugate indicates the binding of the hapten at sites adjacent to the idiotope. The immunogenicity of cellobiose has previously been observed by Gleich and Allen [25]. The IgG antibodies produced when cellobiose is a hapten on a protein carrier, BSA, are very different from the response to the cellobiose moiety of AgB. The anticellobiose antibodies produced by Gleich and Allen [25] cross-reacted significantly with both  $\beta$ -1,3- and  $\beta$ -1,6-diglucosides. In contrast, the anticellobiose reactivity to the AgB determinant is restricted to the  $\beta$ -1,4-diglucoside only. In addition, cellobiose and other  $\beta$ -1,4 sugars are very susceptible to cellulase and other  $\beta$ -1,4 carbohydrases, but the cellobiose moiety of AgB is very resistant to any of the  $\beta$ -1,4 carbohydrases.

Previously, we have shown that our rabbit anti-AgB-specific IgE (anti-Id) does not influence ovalbumin, *Ascaris suum* or keyhole limpet hemocyanin responses in several mouse strains [26]. The specificity of our anti-Id was further demonstrated by its lack of reactivity with mouse IgG, kappa or lambda light chains, NMS, FCS or 2 monoclonal anti-TNP-specific IgE antibodies in an ELISA. The anti-Id gives positive reactions with murine AgB-specific T helper factor, T suppressor factor and IgE in an ELISA. In addition, our anti-Id showed significant inhibition of human timothy-specific IgE binding to WST-coated microtiter wells, but had no effect upon ragweed pollen binding with human antiragweed IgE antibodies (fig. 3).

The structural characterization of the antigenic determinant of AgB showed a requirement of both cellobiose and quercetin moieties to initiate optimal histamine release from monkey lung fragments passively sensitized with serum from timothy-sensitive patients [21, 22]. Thus, the binding of human timothy-specific IgE directed against AgB with anti-Id was not totally unexpected. However, that a large percentage of the serum samples tested were blocked by anti-Id suggests that many patients allergic to timothy grass pollen make a significant part of their total antitimothy IgE antibody against this cellobiose determinant. The relationship of the idiotypic determinants expressed on human AgB-specific IgE and IgG antibodies has not been established. Several recent studies have reported

that the idiotypic determinants expressed on the IgG and IgE antibodies directed against rye group 1 and house dust mite allergens are shared [14, 15]. Our studies with murine AgB-specific IgE and IgG have shown that our anti-Id (made against AgB-specific IgE) will regulate AgB-specific IgE but has no effect upon AgB-specific IgG responses [27]. More recently, we have unsuccessfully tried to inhibit OX-AgB and monoclonal anti-AgB IgG antibody interaction with anti-Id (fig. 2). These observations suggest that in mice the idiotypic determinants expressed on AgB-specific IgE and IgG are distinct, and the same may be true of human AgB-specific IgG and IgE antibodies.

The primary epitope on AgB recognized by AgB-specific IgE is the disaccharide, cellobiose. Recently, Emmrich et al. [28] demonstrated an isotype restriction of idiotype expression on human IgG and IgM antibodies directed against streptococcal A carbohydrate antigens. Thus, the isotype-restricted expression of idiotypic determinants on AgB-specific IgG and IgE antibodies may be due to the carbohydrate nature of the determinant inducing AgB-specific IgE. Alternatively, it may reflect (1) preferential association of certain V genes with either  $C_e$  and  $C_{\gamma 1}$ , or (2) the loss of idiotype expression and generation of new specificities due to somatic mutations. The idiotype-isotype restriction reported here for AgB-specific IgE has also been reported for other antigens among murine IgG subclasses [29].

Previously, we have reported that AgD blocks antigen-induced lymphocyte proliferation of human lymphocytes from timothy-sensitive patients [18, 23]. Cultures of peripheral blood lymphocytes from timothy-sensitive patients with either AgB or anti-Id show significant levels of  $^3\text{H}$ -thymidine incorporation. On the other hand, peripheral blood lymphocytes from either nonallergic individuals or nongrass-allergic patients do not show levels of  $^3\text{H}$ -thymidine incorporation distinct from cultures of cells without antigen or anti-Id (fig. 4). These studies strongly suggest that the idiotypic determinant common to murine AgB-specific T and B cells is also expressed on human T cells of timothy-sensitive patients. The presence of shared idiotypic determinants on a significant number of human T and B cells of timothy-sensitive patients suggests that a public idiotypic determinant is being recognized by the AgB-specific anti-Id. Our observations and the recent findings by Bose et al. [14] and Saint-Rémy et al. [15] regarding the shared idiotypic

determinants expressed on allergen-specific antibodies raise some interesting questions concerning the role of idiotype network perturbations in regulating the formation of human IgE production and the use of idiotype or anti-idiotype reagents in the treatment of allergic patients.

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