

Superantigen Presentation by Airway Smooth Muscle to CD4⁺ T Lymphocytes Elicits Reciprocal Proasthmatic Changes in Airway Function¹

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Microbial products serving as superantigens (SAGs) have been implicated in triggering various T cell-mediated chronic inflammatory disorders, including severe asthma. Given earlier evidence demonstrating that airway smooth muscle (ASM) cells express MHC class II molecules, we investigated whether ASM can present SAG to resting CD4⁺ T cells, and further examined whether this action reciprocally elicits proasthmatic changes in ASM responsiveness. Coincubation of CD4⁺ T cells with human ASM cells pulsed with the SAG, staphylococcal enterotoxin A (SEA), elicited adherence and clustering of class II and CD3 molecules at the ASM/T cell interface, indicative of immunological synapse formation, in association with T cell activation. This ASM/T cell interaction evoked up-regulated mRNA expression and pronounced release of the Th2-type cytokine, IL-13, into the coculture medium, which was MHC class II dependent. Moreover, when administering the conditioned medium from the SEA-stimulated ASM/T cell cocultures to isolated naive rabbit ASM tissues, the latter exhibited proasthmatic-like changes in their constrictor and relaxation responsiveness that were prevented by pretreating the tissues with an anti-IL-13 neutralizing Ab. Collectively, these observations are the first to demonstrate that ASM can present SAG to CD4⁺ T cells, and that this MHC class II-mediated cooperative ASM/T cell interaction elicits release of IL-13 that, in turn, evokes proasthmatic changes in ASM constrictor and relaxant responsiveness. Thus, a new immuno-regulatory role for ASM is identified that potentially contributes to the pathogenesis of nonallergic (intrinsic) asthma and, accordingly, may underlie the reported association between microbial SAG exposure, T cell activation, and severe asthma. *The Journal of Immunology*, 2007, 178: 3627–3636.

Airway sensitization to an airborne Ag is initiated by stimulation of naive CD4⁺ helper T (Th) lymphocytes by professional APCs residing in the airway mucosa (notably, dendritic cells) that process and present the antigenic peptide in association with MHC class II molecules to the TCR. Expression of MHC class II molecules, however, is not restricted to professional APCs and is seen in various parenchymal cell types including endothelial cells, epithelial cells, keratinocytes, and fibroblasts (1–3). Although inherently not involved in Ag processing, these cells can elicit T cell activation by presenting superantigen (SAG)³ as unprocessed protein associated with MHC class II molecules to the TCR at sites within its β variable ($V\beta$) region,

which are distinct from the TCR regions involved in cognate recognition of the MHC molecule (4, 5). SAGs are produced by bacteria or viruses and have been implicated in the etiopathology of various inflammatory diseases, including atopic disorders and severe asthma (6–11). In relation to atopic disease, polyclonal T cell activation with the SAG, staphylococcal enterotoxin B (SEB), was found to be associated with induced release of the Th2-type cytokines, IL-4 and IL-5, and reduced release of the Th-1 cytokine, IFN- γ , from PBMCs isolated from atopic individuals (12–15). Although this Th2-skewed pattern of cytokine release is characteristic of atopic diseases, including allergic asthma, it is noteworthy that airway exposure to SEB was also found to elicit CD4⁺ T cell-dependent airway inflammation accompanied by intrapulmonary IL-4 and TNF- α production and airway hyperresponsiveness in a murine model of nonallergic asthma (16). Thus, when taken together, these previous findings indicate that SAG-induced CD4⁺ T cell activation can be seen both under atopic and nonatopic predisposing conditions, and that the airway effects of SAG exposure, including the induction of proinflammatory cytokine production, changes in airway responsiveness, and asthma may be evidenced independent of any preexisting Ag-specific airway sensitization. The mechanism underlying the SAG-induced effects on airway function, however, remains to be identified.

In considering potential mechanisms responsible for induction of the asthmatic phenotype of altered airway responsiveness, it is relevant to note that, beyond its inherent role as a regulator of airway tone, the airway smooth muscle (ASM) was found to directly respond to various stimuli (e.g., IgE immune complexes, certain viral pathogens, and specific aeroallergens) that can elicit proasthmatic-like changes in its constrictor and relaxation responsiveness (17–20). Additionally, recent studies have demonstrated

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³ Abbreviations used in this paper: SAG, superantigen; SEA, staphylococcal enterotoxin A; ASM, airway smooth muscle; ACh, acetylcholine; Tmax, maximal isometric contractile force; Rmax, maximal isometric relaxation; MFI, mean fluorescence intensity.

that activated human ASM cells and CD4⁺ T cells in coculture exhibit direct bidirectional stimulatory cross-talk, and that this intercellular communication is mediated by coligation of complementary cell surface adhesion and costimulatory molecules expressed by both cell types (21, 22). Furthermore, this ASM/T cell costimulation was found to be associated with the evoked release of proinflammatory cytokines, the latter resulting in the induction of proasthmatic-like changes in ASM responsiveness (22); and ASM/T cell interaction was also found to induce ASM proliferation, a characteristic feature of airway remodeling in asthma (23, 24). Thus, given this evidence of direct ASM/T cell communication, together with the finding that stimulated ASM cells also exhibit enhanced cell surface expression of MHC class II molecules (21, 25), this study addressed the interrelated hypotheses that ASM cells can serve as accessory cells for SAg presentation and activation of resting nonatopic/nonasthmatic CD4⁺ T cells, and that this action elicited by SAg-presenting ASM is accompanied by reciprocal induced changes in ASM responsiveness. The results provide new evidence demonstrating that: 1) cultured human ASM cells are capable of presenting the SAg, staphylococcal enterotoxin A (SEA), via their MHC class II molecules to resting CD4⁺ T cells, forming an immunological synapse, and thereby evoking T cell activation; and 2) this MHC class II-mediated cooperative ASM/T cell interaction elicits release of the Th2-type proinflammatory cytokine, IL-13, that, in turn, evokes proasthmatic-like changes in the constrictor and relaxant responsiveness of isolated naive ASM tissues. Collectively, these observations support the novel concept that, independent of any preexisting airway sensitization associated with the adaptive immune response that underlies allergic asthma, or any specific preexisting bias of adaptive immunity, ASM can directly provoke a proasthmatic nonallergic airway immune response by presenting SAg to CD4⁺ T lymphocytes. In this context, the present findings are the first to identify an innate role for ASM in potentially mediating the reported association between microbial superantigen exposure, T cell activation, and severe asthma (9).

Materials and Methods

Reagents

SEA, IFN- γ , and all chemicals used were purchased from Sigma-Aldrich unless otherwise indicated. The human ASM cells were obtained from BioWhittaker, Ham's F-12 medium was purchased from Mediatech, and 10% FBS was obtained from HyClone Laboratories. The mouse anti-human monoclonal MHC class II (HLA-DR) neutralizing Ab (clone G46-6) and the anti-CD54 monoclonal blocking Ab were purchased from BD Biosciences, and the mouse monoclonal anti-human IL-13 neutralizing Ab and the human IL-13 ELISA kit were obtained from R&D Systems. Alexafluor 568-conjugated highly cross-adsorbed goat anti-mouse Ab was obtained from Molecular Probes/Invitrogen.

Isolation of human PBMCs and CD4⁺ T cells

PBMCs were isolated from freshly drawn whole blood samples from normal subjects having no history of allergic disease or asthma by Ficoll-Hypaque density gradient centrifugation. CD4⁺ T cells were isolated and enriched (typically >95% purity) using the RosetteSep CD4 reagent from StemCell Technologies. The isolated PBMCs and CD4⁺ T cells were subsequently exposed to the cultured ASM cell preparations in a concentration of 1×10^6 to 5×10^6 cells/ml under different experimental conditions, as described below. Freshly isolated cells were consistently used within 1 h of their preparation.

Animals

Eighteen adult New Zealand White rabbits were used in this study, which was approved by the Biosafety and Animal Research Committee of the Joseph Stokes Research Institute at Children's Hospital of Philadelphia. The animals had no signs of respiratory disease for several weeks before the study, and their care and use were in accordance with the Guide for the

Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council.

ASM cell culture and cocubation with PBMCs and T cells

The human ASM cells were derived from a young adult male who had no evidence of pulmonary or cardiovascular disease. The cells were grown in Ham's F-12 medium supplemented with 10% FBS, maintained throughout in a humidified incubator containing 5% CO₂ in air at 37°C, and used for study after 5–6 passages in culture. The experimental protocol involved growing the cells to 95% confluence in the above medium. Thereafter, in separate experiments, the cells were starved in unsupplemented Ham's F-12 medium for 24 h, and then treated with either vehicle alone, varying concentrations of IFN- γ (5–250 ng/ml) for 72 h to enhance their MHC class II expression, varying concentrations of SEA (.05–1 μ g/ml) for 24 h, or IFN- γ for 72 h initially followed by SEA exposure for an additional 24 h. Isolated PBMC or CD4⁺ T cell preparations were then introduced into the ASM cell cultures for varying durations indicated. Subsequently, the PBMCs or T cells and the ASM cells were separated by rinsing three times of the cocultures, as previously described (21, 22), and the isolated PBMCs or T cells and ASM cells were prepared for either flow cytometric analysis or mRNA detection of cytokine expression. Verification of effective removal of the T cells was demonstrated by their absence when staining the cell preparation with an anti-CD3 Ab (21, 22). Additionally, at indicated times following cocubation of ASM cells with T cells under the different experimental conditions, aliquots of the coculture medium were collected for assay of IL-13 protein concentration using an ELISA kit.

Flow cytometry

Cell surface expression of the MHC class II Ag, HLA-DR, and CD54 (ICAM-1) was examined in untreated and in IFN- γ -pretreated ASM cells by flow cytometric analysis following their nonenzymatic cellular dissociation. Cell surface expression of CD3, CD4, and CD69 was examined in PBMCs, and CD25 was detected in isolated CD4⁺ T cells, both in the absence and presence of cocubation of the cells with ASM cells under the above treatment conditions. As previously described (21), cells were prepared and stained with PE-conjugated mouse anti-human primary mAbs directed against HLA-DR, CD54, and CD69, FITC-conjugated mAb directed against CD25, PerCP-conjugated mAb directed at CD3, and allophycocyanin-conjugated mAb directed at CD4. Cells were also stained with mouse or rat IgG Abs of the identical isotypes as the above primary mAbs to assess for background fluorescence. The flow cytometric analyses were performed using a BD Bioscience flow cytometer equipped with a 15 mW argon ion laser operated at 488 nm, and the data analyzed using the CellQuest-Pro software (BD Bioscience). Fluorescence signals were accumulated as two-parameter fluorescence histograms with both percent positive cells and mean channel fluorescence intensity (MFI) being recorded.

Microscopic evaluation of ASM/T cell contact

ASM cells were initially grown to 50–60% confluence on 1.7 cm² BD-Falcon culture well microscope slides, subsequently starved in unsupplemented Ham's F-12 medium for 24 h, and then treated for 72 h with either medium alone (control), IFN- γ (250 ng/ml), or IFN- γ together with SEA (1 μ g/ml). Thereafter, the slides were rinsed three times with PBS and 5×10^5 freshly isolated CD4⁺ T cells were added to each slide in a total of 100 μ l RPMI 1640 containing 10% heat-inactivated FBS and incubated in a humidified chamber with 5% CO₂ at 37°C for 30 min. After removing nonadherent CD4⁺ T cells by gentle rinsing with PBS, remaining cells were fixed with 3.7% formaldehyde for 15 min at room temperature. Cells were then incubated with mouse anti-human HLA-DR Ab for 1 h at room temperature, rinsed three times with PBS containing 1% BSA and then incubated with highly cross-adsorbed AlexaFluor 568-conjugated goat anti-mouse for 1 h at room temperature. Slides were then rinsed again with PBS containing 1% BSA and 1.5 mm glass coverslips were mounted using Prolong-Antifade reagent (Molecular Probes/Invitrogen). To visualize CD3, cells were incubated with FITC-conjugated CD3 ϵ (BD Biosciences) instead of the anti-HLA-DR and anti-mouse combination. Slides were imaged using an Olympus IX-81 epifluorescent microscope (60 $\times$ objective) with disc spinning unit to achieve confocality. Images were processed using Slidebook software (Intelligent Imaging Innovations). The number of T cells adhering to an ASM cell was evaluated using differential interference contrast in 3–4 independent samples. An average of 51 ASM cells was evaluated per repeat.

Detection of IFN- γ and IL-13 mRNA transcripts

CD4⁺ T cells and ASM cells were cocubated for varying durations up to 24 h under different experimental conditions. Total RNA was extracted

from the separated T cells and ASM cells using the TRIzol method, and cDNAs were isolated by RT-PCR using the SuperScript first strand synthesis system kit from Invitrogen (Life Technologies), with the following IFN- γ - and IL-13-specific oligonucleotide primer sets purchased from Integrated DNA Technologies: for IFN- γ , 5'-TGACCAGAGCATCCAAA GA-3' (forward); 5'-GCATCTGACTCTTTTCGC-3' (reverse); and for IL-13, 5'-CATGGCGCTTTTGTGACCA-3' (forward); 5'-CATCCTCTGGTCTTCTCGA-3' (reverse). The reaction volume was 20 μ l and cycling conditions used were 35 cycles of 30 s denaturation at 95°C, followed by 30 s annealing at 60°C and elongation at 72°C for 30 s. Ex-Tag (Takara Biotechnology) was used as DNA polymerase.

Preparation and treatment of rabbit ASM tissues

Following initial sedation and subsequent general anesthesia with i.m. injections of xylazine (10 mg/kg) and ketamine (50 mg/kg), respectively, rabbits were sacrificed with an i.v. administered overdose of pentobarbital (125 mg/kg). As described previously (19), the tracheae were removed via open thoracotomy, the loose connective tissue and epithelium were scraped and removed, and the tracheae were divided into 8 ring segments, each of 6–8 mm in length. The airway segments were then placed in modified Krebs-Ringer solution containing indomethacin (10 μ M), and each alternate ring was incubated for 24 h at room temperature in the presence of either vehicle alone (control) or conditioned medium collected after 5 days of coculture of CD4⁺ T cells with human ASM cells under different experimental conditions, both in the absence and presence of pretreatment of the rabbit ASM tissues with either an anti-IL-13 neutralizing mAb (1 μ g/ml) or a corresponding mouse IgG1 isotype control Ab. All the tissues studied were continuously aerated with a gas mixture containing 95% O₂ and 5% CO₂ throughout the incubation phase.

Pharmacodynamic studies of ASM constrictor and relaxant responsiveness

Following incubation, the tissues were placed in organ baths containing modified Krebs-Ringer solution aerated with 5% CO₂ in oxygen (pH of 7.35–7.40), and the tissues were attached to force transducers from which isometric tension was continuously displayed on a multichannel recorder, as previously described (19). Cholinergic contractility was then assessed in the ASM segments by cumulative administration of acetylcholine (ACh) in final bath concentrations ranging from 10^{−9} to 10^{−3} M. Thereafter, the tissues were repeatedly rinsed with fresh buffer and, subsequently, relaxation dose-response curves to cumulatively administered isoproterenol (10^{−9}–10^{−4} M) were generated after the tissues were half-maximally contracted with their respective ED₅₀ doses of ACh. The initial constrictor dose-response curves to ACh were analyzed with respect to each tissue's maximal isometric contractile force (T_{max}) to the agonist; the subsequent relaxation responses to isoproterenol were analyzed in terms of percentage of maximal relaxation (R_{max}) from the initial level of active cholinergic contraction.

Statistical analysis

The results are expressed as mean $\pm$ SE values. Comparisons between groups were made using the Student's *t* test (two-tailed) or ANOVA with Tukey's posttest analysis, where appropriate. A probability of < 0.05 was considered statistically significant. Statistical analyses were conducted using the Prism computer program by GraphPad Software.

Results

CD4⁺ T cell activation by SAg-presenting ASM cells

Although previously shown to express cellular adhesion and costimulatory molecules that mediate T cell activation, ASM cells are not obligatory APCs, and they display a relatively low density of MHC class II molecules on their cell surface under resting conditions (21, 25). To test their potential to present SAg to resting T cells, confluent cultures of ASM cells were initially exposed for 3 days to IFN- γ , a treatment condition that characteristically up-regulates cell surface expression of MHC class II and intercellular adhesion molecules in a variety of cell types including ASM cells (1, 25, 26). As exemplified by the flow cytometric tracings in Fig. 1A, relative to the low level of expression of the class II Ag, HLA-DR, detected in control (vehicle-exposed) cells, ASM cells stimulated with a maximally effective concentration of IFN- γ (150 ng/ml) exhibited enhanced cell surface expression of HLA-DR,

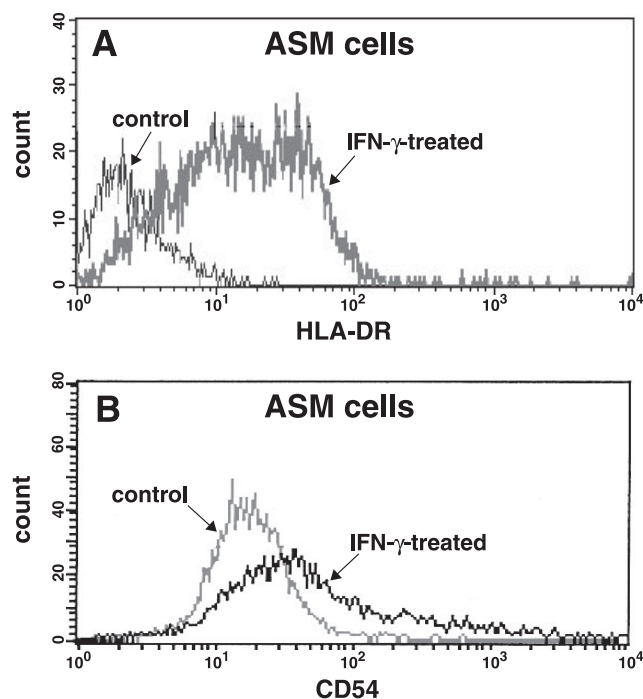
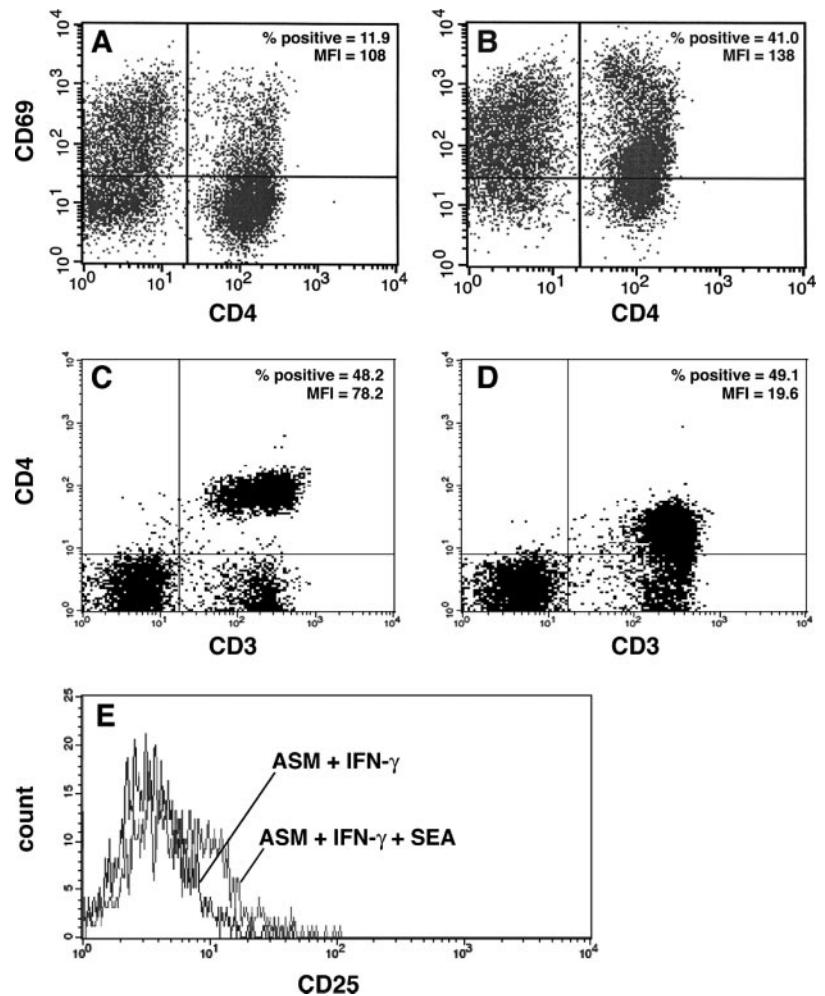


FIGURE 1. Enhanced cell surface expression of HLA-DR (A) and CD54 (B) in IFN- γ -treated cultured human ASM cells. Confluent ASM cell cultures were treated for 3 days with IFN- γ (150 ng/ml), and subsequently suspended cells were stained with PE-conjugated mouse anti-human primary mAbs directed against HLA-DR and CD54. Representative flow cytometric tracings demonstrate that, relative to control (vehicle-exposed) cells, IFN- γ -treated ASM cells exhibited enhanced cell surface expression of HLA-DR and CD54. Data from 3 experiments demonstrated significant increases ($p < 0.01$) in both % positive cell staining and MFI for HLA-DR that averaged 2.8- and 8.7-fold, respectively, above the corresponding values obtained in control cells. The IFN- γ -treated ASM cells also displayed significant increases in MFI for CD54 expression that averaged 3.6-fold above the levels detected in control cells ($p < 0.01$). Note: levels of nonspecific background staining were also measured using PE-conjugated isotype control IgG Abs, and there were no differences in nonspecific background staining for either HLA-DR or CD54 between control and IFN- γ -treated conditions (data not shown).

with significant increases in both percentage of positive cell staining and MFI that averaged 2.8- and 8.7-fold above the corresponding values obtained in control cells. Moreover, as exemplified in Fig. 1B, the IFN- γ -treated ASM cells also displayed up-regulated cell surface expression of ICAM-1 (CD54), with significant increases in MFI ($p < 0.01$) that averaged 3.6-fold above the levels detected in control cells.

Given the above effects of IFN- γ , activation of resting CD4⁺ T cells by SAg presentation was then assessed under different experimental conditions, including in the absence and presence of their cocubation with ASM cells that were pretreated for 3 days with IFN- γ and then pulsed for 24 h with a maximally effective concentration of SEA (250 ng/ml). Flow cytometric studies were initially conducted using isolated PBMCs to examine the cellular specificity of responses to different conditions of coculture with ASM cells. Accordingly, PBMCs were examined with respect to induced changes in their cell surface expression of CD69 and CD4 following cocubation with SEA-presenting ASM cells. As exemplified by the dual-parameter fluorescence histograms for CD4-stained PBMCs in Fig. 2, compared with cells directly exposed to IFN- γ plus SEA in the absence of ASM cells (Fig. 2A), PBMCs that were exposed to IFN- γ -pretreated/SEA-pulsed ASM cells exhibited increased CD69 expression (Fig. 2B). This enhanced CD69

FIGURE 2. Activation of CD4⁺ T cells by SEA-presenting ASM cells. *Upper panels:* Representative two-parameter fluorescence histograms of isolated PBMCs demonstrating that, relative to cells that were directly exposed to IFN- γ plus SEA in the absence of coincubated ASM cells (**A**), CD69 expression was most notably increased in the CD4⁺ cells when coincubating the PBMCs with IFN- γ -pretreated plus SEA-pulsed ASM cells (**B**). *Middle panels:* Two-parameter fluorescence histograms demonstrating that, relative to PBMCs cocultured with ASM cells that were pretreated with IFN- γ alone (**C**), CD4 expression was distinctly reduced in PBMCs that were cocultured with IFN- γ -pretreated plus SEA-pulsed ASM cells (**D**). *Lower panel (E):* Representative single-parameter histograms of isolated CD4⁺ T cells demonstrating that, relative to coincubation with ASM cells pretreated with IFN- γ alone, CD25 expression was enhanced when exposing the T cells to IFN- γ -pretreated plus SEA-pulsed ASM cells. Note: levels of nonspecific background staining detected using isotype control IgG Abs showed no differences in nonspecific background staining when comparing the different treatment conditions (data not shown).



expression was relatively more pronounced in the CD4⁺ cells, wherein the values for percentage of positive cell staining and MFI were increased by ~ 3.5 - and 1.3-fold, respectively, as compared with the corresponding increases in percentage of positive cell staining and MFI of ~ 1.3 - and 1.1-fold, respectively, detected in the CD4-negative cells. CD4⁺ T cell activation by SEA-presenting ASM cells was also observed when examining for changes in cell surface expression of CD4 on CD3-stained PBMCs. Relative to PBMCs exposed to ASM cells that were pretreated with IFN- γ alone (Fig. 2C), the MFI for CD4⁺ staining was markedly reduced by $\sim 75\%$ in PBMCs that were coincubated with IFN- γ -pretreated/SEA-pulsed ASM cells (Fig. 2D). In light of this evidence of activation of CD4⁺ cells by SEA-presenting ASM cells, extended studies were conducted using isolated CD4⁺ T cells, wherein we examined for induced changes in cell surface expression of CD25, another early marker of CD4⁺ T cell activation. As shown in Fig. 2E, relative to CD4⁺ T cells exposed to ASM cells that were pretreated with IFN- γ alone, wherein percentage of positive cell staining for CD25 amounted to 5.9% (MFI = 12.3), percentage of positive cell staining was significantly enhanced to 27.9% (MFI = 13.9) in T cells that were coincubated with IFN- γ -pretreated ASM cells that were pulsed with SEA. Collectively, these observations demonstrate that IFN- γ -pretreated ASM cells can effectively present SEA to resting CD4⁺ T cells and, thereby, evoke T cell activation.

Direct ASM cell interaction with CD4⁺ T cells

The nature of the interaction between cocultured CD4⁺ T cells and ASM cells was further evaluated microscopically. In these studies,

freshly isolated CD4⁺ T cells were incubated for 30 min with ASM cells that were either exposed to vehicle alone (control), pretreated with IFN- γ , or IFN- γ -pretreated and pulsed with SEA. Nonadherent T cells were removed by rinsing and the residual attached CD4⁺ T cells were enumerated. As depicted in Fig. 3A, relative to their respective controls, ASM cells pretreated with IFN- γ displayed a ~ 2.7 -fold increase in the average number of CD4⁺ T cells bound per ASM cell. A similar degree of enhanced T cell adhesion was detected in IFN- γ -pretreated ASM cells that were pulsed with SEA, and this induced adhesion was inhibited by pretreating the ASM cells either with an anti-ICAM-1 blocking mAb (10 $\mu\text{g}/\text{ml}$) or with an anti-MHC class II neutralizing mAb (10 $\mu\text{g}/\text{ml}$). Moreover, in relation to these observations, it should be noted that $21 \pm 1\%$ of ASM cells that were pretreated with IFN- γ alone displayed 3 or 4 adherent T cells and, of ASM cells that were pretreated with IFN- γ and pulsed with SEA, a similar percentage ($21.3 \pm 3\%$) showed 3 or more adherent T cells. Thus, in concert with their IFN- γ -induced up-regulated expression of ICAM-1 and HLA-DR (Fig. 1), IFN- γ -pretreated ASM cells that were pulsed with SEA exhibited both ICAM-1- and MHC class II-dependent T cell adherence. These observations are consistent with the previously reported role of ICAM-1 in mediating stimulated ASM cell/T cell adhesion (21, 24), as well as with the established role of MHC class II/TCR engagement in triggering T cell adhesion to APCs by inducing coligation of LFA-1 with ICAM-1 in association with immunological synapse formation (27).

Our observed activation of CD4⁺ T cells coupled to their adherence to SEA-pulsed ASM cells implies the induction of specific TCR stimulation by the SEA-presenting ASM cells. To evaluate

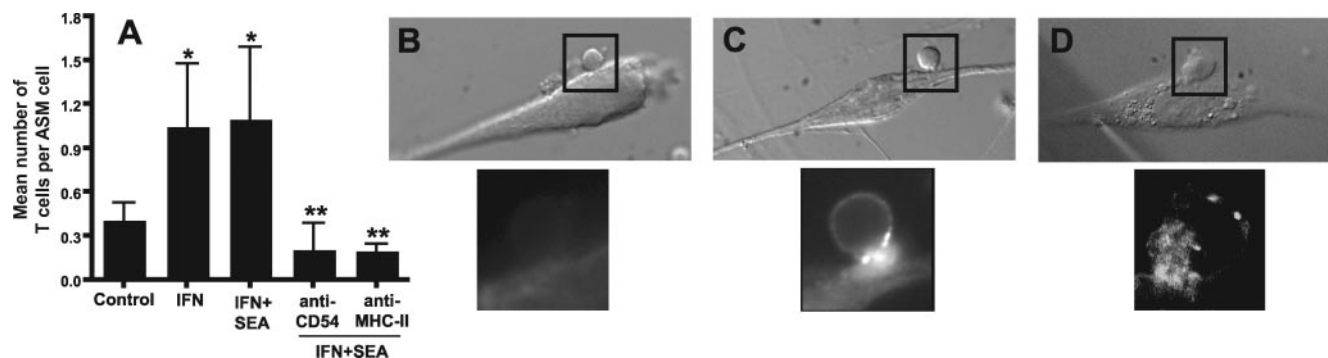


FIGURE 3. Direct interaction between $CD4^+$ T cells and ASM cells. (A) Comparison of adherence of freshly isolated $CD4^+$ T cells to monolayers of ASM cells under control (unstimulated) conditions and accompanying coincubation of the T cells with ASM cells that were pretreated with IFN- γ alone or with IFN- γ -pretreated plus SEA-pulsed ASM cells in the absence and presence of blocking Abs directed against CD54 (ICAM-1) or MHC class II. T cell/ASM cell adhesion was determined after 30 min of coincubation at 37°C. A mean of 51 ASM cells was examined over 3–6 repeats and adhesion is expressed as the mean number of T cells bound per ASM cell. Relative to control, the increase in T cell adherence to IFN- γ treated ASM cells and to IFN- γ -pretreated plus SEA-pulsed ASM cells was significant (* = $p < 0.01$). In IFN- γ -pretreated plus SEA-pulsed ASM cells, the inhibition of adherence obtained in the presence of blocking Abs was also significant (** = < 0.01). (B–D) Representative T cell adhesion to ASM cells as evaluated by DIC microscopy, with distribution of HLA-DR (B, C) and CD3 (D) as discerned by spinning disc confocal microscopy at the T cell/ASM cell interface in the region defined by the black square (bottom panels). As compared with adherence to ASM pretreated with IFN- γ alone (B), T cell adherence to IFN- γ -pretreated plus SEA-pulsed ASM cells was associated with distinct clustering of HLA-DR (C) and CD3 (D) at the ASM/T cell interface.

whether this phenomenon is associated with the formation of immunological synapses between the ASM cell and T cell conjugates, the latter were examined for evidence of localization of HLA-DR and CD3 molecules at the ASM cell/T cell interface. As exemplified by the fluorescent micrographs in Fig. 3, compared with cocultures wherein ASM cells were pretreated with IFN- γ alone (Fig. 3B), cocultures containing IFN- γ -pretreated ASM cells that were pulsed with SEA displayed distinct clustering of both HLA-DR molecules (Fig. 3C) and CD3 molecules (Fig. 3D) at the ASM/T cell interface. Accordingly, HLA-DR and CD3 clustering was observed in 22% and 24% of ASM/T cell conjugates involving SEA-pulsed ASM cells, respectively, as compared with <4% of conjugates involving ASM cells that were treated with IFN- γ alone. Thus, whereas pretreatment of ASM cells with IFN- γ alone was found to produce ASM/T cell adhesion, pulsing the IFN- γ -pretreated ASM cells with SEA evoked HLA-DR and CD3 clustering at the ASM/T cell interface, a characteristic feature of immunological synapse formation.

Induced changes in ASM responsiveness in the SEA-conditioned state

In light of the above evidence demonstrating $CD4^+$ T cell activation by SEA-presenting ASM cells, we next conducted a series of studies to systematically investigate whether this phenomenon evokes reciprocal induced changes in ASM function. In addressing this issue, we initially examined whether coincubation of $CD4^+$ T cells with SEA-presenting human ASM cells elicits release of a bronchoactive factor(s) into the coculture medium that, when administered to isolated naive rabbit ASM tissues, produces changes in the tissues' constrictor and relaxation responsiveness. Accordingly, agonist-induced constrictor and relaxation responses were compared in control (vehicle-treated) rabbit ASM tissues and tissues exposed for 24 h to conditioned medium collected after 5 days of coincubation of $CD4^+$ T cells with human ASM cells under different experimental conditions. As shown in Fig. 4A, relative to control tissues (open circles), ASM segments exposed to medium from T cells cocultured with ASM cells pretreated with SEA alone (open squares) exhibited similar constrictor responses to exogenously administered ACh. Contrasting with this observation, ASM tissues that were exposed to the conditioned medium from T

cells coincubated with IFN- γ -pretreated ASM cells that were subsequently pulsed with SEA, henceforth referred to as SEA-conditioned medium, exhibited increased constrictor responsiveness to ACh, wherein the mean $\pm$ SE maximal constrictor response (T_{max}) amounted to 110.2 ± 10.6 g/g ASM wt., which was significantly greater ($p < 0.05$) than the corresponding mean T_{max} response obtained in control tissues (i.e., 91.9 ± 6.9 g/g ASM wt.). As further depicted in Fig. 4A (open triangles), this increased constrictor responsiveness to ACh was completely abrogated in ASM tissues that were exposed to SEA-conditioned medium wherein the cocultured ASM cells were initially treated for 30 min with an anti-MHC class II neutralizing mAb (10 μ g/ml) before exposing the cells to SEA. By comparison, there was no effect on the induced changes in constrictor responsiveness in ASM tissues exposed to the SEA-conditioned medium wherein the cocultured ASM cells were pretreated with the same concentration of an isotype control IgG2 α Ab (cAb) (filled triangles).

Under the same treatment conditions, during subsequent sustained half-maximal contraction with ACh, administration of the β -adrenoceptor agonist, isoproterenol, produced cumulative dose-dependent relaxation of the preconstricted ASM segments. As shown in Fig. 4B, similar relaxation responses to isoproterenol were generated in control ASM tissues and in tissues exposed to medium from T cells cocultured with ASM cells that were pretreated with SEA alone. Contrasting with this observation, the relaxation responses were significantly attenuated in the tissues exposed to the SEA-conditioned medium. Accordingly, relative to the mean $\pm$ SE maximal relaxation (R_{max}) response of $48.2 \pm 3.2\%$ obtained in the control ASM segments, the R_{max} responses in the tissues exposed to the SEA-conditioned medium averaged $28.1 \pm 4.1\%$ ($p < 0.01$). This impaired relaxation responsiveness to isoproterenol was prevented in ASM tissues that were exposed to SEA-conditioned medium wherein the cocultured ASM cells were initially treated with the anti-MHC class II neutralizing mAb, whereas there was no effect on the induced changes in relaxation responsiveness in ASM tissues exposed to SEA-conditioned medium wherein the cocultured ASM cells were pretreated with the isotype control Ab (filled triangles).

In relation to these observations, it should be noted that, in comparable experiments conducted on ASM tissues that were directly

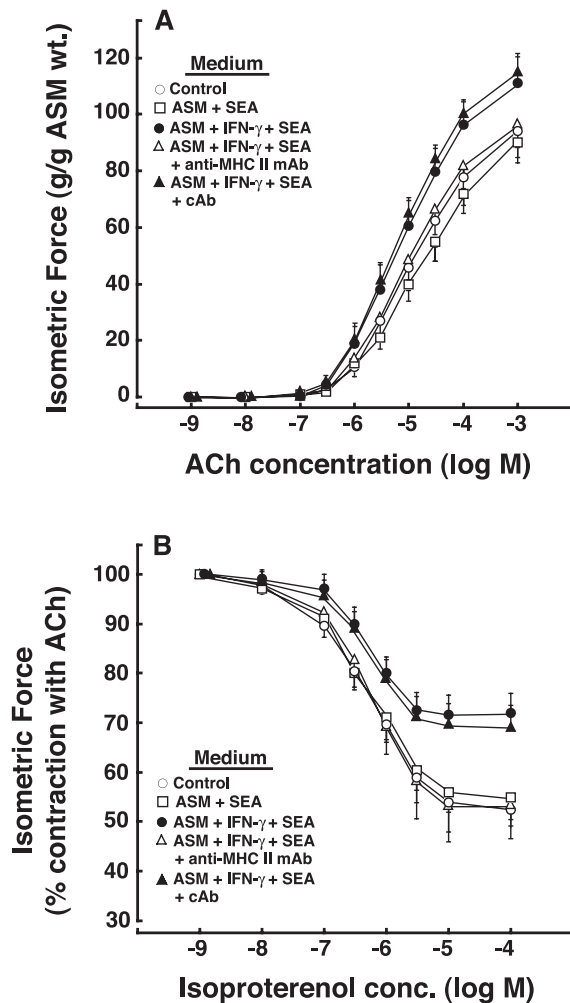


FIGURE 4. Proasthmatic-like changes in constrictor and relaxation responsiveness in rabbit ASM tissues exposed to conditioned medium from cocultured CD4⁺ T cells and SEA-presenting ASM cells. (A) Comparison of constrictor dose-response relationships to ACh obtained in isolated naive rabbit ASM tissue segments exposed for 24 h to medium from either control (vehicle-treated) ASM cell/T cell cocultures (open circles), cocultures containing ASM cells initially exposed to SEA alone (open squares), and cocultures containing IFN- γ -treated plus SEA-pulsed ASM cells both in the absence (filled circles) and presence of pretreatment of the cocultured ASM cells with either an anti-MHC class II blocking mAb (open triangles) or a corresponding isotype control Ab (filled triangles). (B) Comparison of the corresponding relaxation dose-response relationships to isoproterenol generated in the same ASM tissue segments following their half-maximal contraction with ACh. Note: relative to their respective controls, the heightened T_{max} responses to ACh and impaired R_{max} responses to isoproterenol obtained in tissues exposed to the conditioned medium from cocultures containing IFN- γ -treated plus SEA-pulsed ASM cells were ablated when the cocultured ASM cells were pretreated with the class II blocking mAb. Data represent mean $\pm$ SE values from six paired experiments.

exposed to either SEA or IFN- γ alone, or in combination, or to medium from T cells cocultured with ASM cells that were pretreated with IFN- γ alone (i.e., not pulsed with SEA), we found no effect of either treatment condition on the tissues' constrictor or relaxant responsiveness (data not shown).

Evoked cytokine expression in the SEA-conditioned state

We previously demonstrated that coculture of anti-CD3-activated T cells with human ASM cells elicits cooperative intercellular sig-

naling that evokes expression of proinflammatory cytokines from both cell types (22). This earlier evidence, when considered in light of the present observations, raises the consideration that the above-observed proasthmatic-like changes in ASM responsiveness may be attributed to induced cytokine expression accompanying T cell activation by the SEA-presenting ASM cells. In addressing this issue, we initially examined whether coculture of CD4⁺ T cells with SEA-presenting ASM cells is associated with evoked changes in mRNA expression of the prototypical Th1- and Th2-type cytokines, IFN- γ and IL-13, in both cell types, as changes in expression of these cytokines have been implicated in the pathobiology of asthma (28, 29), as well as in the induction of altered ASM responsiveness (29, 30). RNA was extracted from T cells and ASM cells harvested following their cocultivation for varying durations under different experimental conditions, and PCR were generated using primers to detect both cytokine-specific and constitutively expressed β -actin transcripts. As depicted in Fig. 5A, in contrast to the lack of effect when cocultivating T cells with ASM cells exposed to IFN- γ alone, coculture of T cells with SEA-presenting ASM cells evoked temporal changes in expression of both IFN- γ and IL-13 transcripts, which differed between the two cell types. Accordingly, relative to unaltered expression of β -actin mRNA, T cells exhibited up-regulated expression of IFN- γ transcripts at 6 and 12 h of cocultivation with SEA-presenting ASM cells and, by 24 h, the IFN- γ transcripts were undetectable, whereas IL-13 transcripts were initially detected at 12 h and their up-regulated expression was sustained at 24 h. Contrasting with these observations, the cocultured ASM cells exhibited up-regulated expression of IFN- γ transcripts at 6 h, which was sustained thereafter and, at 12 and 24 h, IL-13 transcripts were concomitantly detected. When taken together, these data demonstrate that, whereas T cells exposed to SEA-presenting ASM cells exhibited a transition from initial induced mRNA expression of IFN- γ to subsequent expression of IL-13, the cocultured ASM cells displayed sustained up-regulated coexpression of transcripts for both these Th1- and Th2-type cytokines. Finally, to substantiate that the induction of altered cytokine mRNA expression accompanying the SEA-stimulated state represented a MHC class II-mediated effect, in extended experiments we examined whether the induced changes in IFN- γ and IL-13 mRNA expression are modulated in the presence of an anti-MHC class II neutralizing mAb. As demonstrated in Fig. 5B, the induced changes in cytokine mRNA expression detected in both cell types accompanying their cocultivation for 24 h in the SEA-presenting condition were largely abrogated when the ASM cells were pretreated with the anti-MHC class II mAb.

In light of the above results, we next examined for induced temporal changes in IL-13 protein release into the ASM cell/T cell coculture medium under various experimental conditions, including in the absence and presence of SEA presentation by the cocultured ASM cells. As shown in Fig. 6, relative to their corresponding basal determinations that averaged 49 ± 21 pg/ml, the IL-13 protein levels detected in the culture medium were generally increased to a variably significant extent on day 2 following exposure of the CD4⁺ T cells to IFN- γ plus SEA in the absence of ASM cells, and following cocultivation of the T cells with ASM cells pretreated with SEA alone; and there were no further significant changes in IL-13 accumulation on the subsequent days of coculture. In contrast, coculture of T cells with ASM cells that were initially treated with IFN- γ and then pulsed with SEA elicited striking temporal increases in IL-13 accumulation in the coculture medium, amounting to 709 ± 65 pg/ml on day 6 of coculture. As further demonstrated, this marked stimulation of IL-13 release was

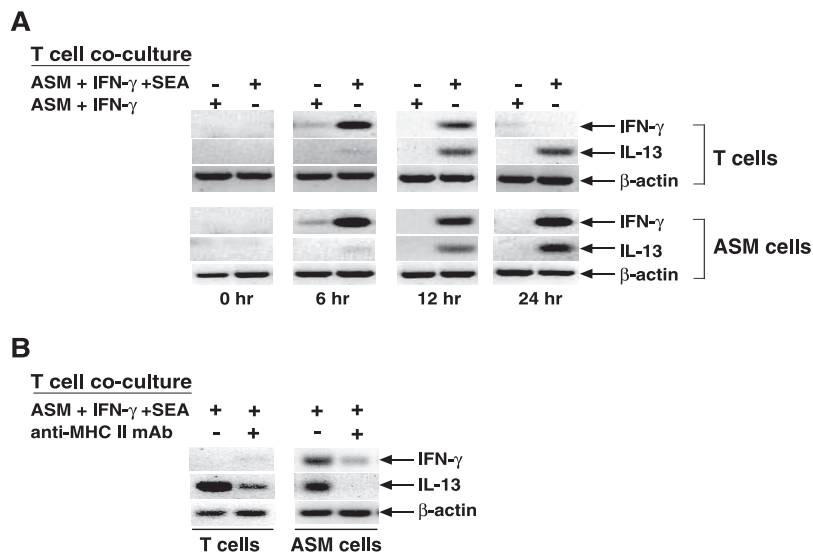


FIGURE 5. Temporal changes in IFN- γ and IL-13 mRNA expression exhibited by both cocultured CD4⁺ T cells and ASM cells accompanying SEA presentation by the cocultured ASM cells. The cytokine transcripts were detected using RT-PCR after 0, 6, 12, and 24 h of cocubation of the T cells either with ASM cells that were pretreated for 3 days with IFN- γ alone or with IFN- γ -treated plus SEA-pulsed ASM cells. Expression of β -actin was used to control for gel loading. The blots were probed with human-specific IFN- γ and IL-13 cDNA probes (see Methods). (**A**): In contrast to undetectable IFN- γ and IL-13 transcripts in the cocubated T cells and ASM cells pretreated with IFN- γ alone, coculture of T cells with IFN- γ -treated plus SEA-pulsed ASM cells evoked initial increased expression of IFN- γ transcripts in both cell types at 6 h and, whereas IFN- γ mRNA expression was subsequently ablated in the T cells at 24 h, enhanced expression of the IFN- γ transcripts was sustained in the cocubated ASM cells. Induced expression of IL-13 transcripts was detected in both the cocubated T cells and IFN- γ -treated plus SEA-pulsed ASM cells at 12 h, and subsequently sustained at 24 h. (**B**): Induced changes in expression of the IFN- γ and IL-13 transcripts detected at 24 h in T cells and in cocubated SEA-presenting ASM cells were prevented by pretreating the SEA-presenting ASM cells with an anti-MHC class II neutralizing mAb (10 μ g/ml). Constitutively expressed β -actin mRNA was similar at all times under the different treatment conditions.

abrogated when the cocubated SEA-pulsed ASM cells were pretreated with an anti-MHC class II neutralizing mAb (i.e., before exposing the ASM cells to IFN- γ plus SEA). Thus, these observations demonstrate that the pronounced induced release of IL-13

into the SEA-conditioned medium was attributed to MHC class II-mediated presentation of the superantigen by the cocubated ASM cells.

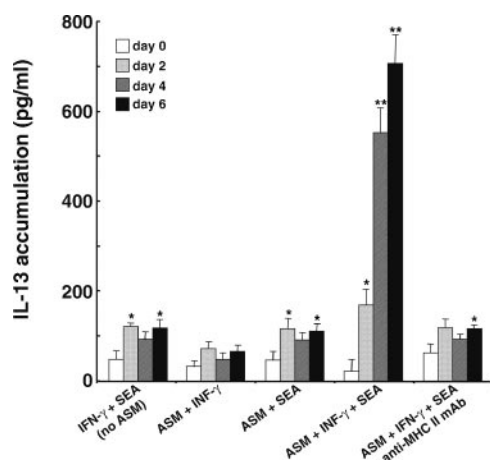


FIGURE 6. Temporal changes in IL-13 protein release elicited in the absence and presence of coculture of CD4⁺ T cells with ASM cells under different pretreatment conditions. Relative to basal determinations, levels of IL-13 accumulation in the culture medium were initially moderately but significantly increased on day 2–3, and sustained thereafter, either by treating T cells with IFN- γ plus SEA directly or by cocubating T cells with ASM cells that were pulsed with SEA. By comparison, coculture of T cells with IFN- γ -treated plus SEA-pulsed ASM cells evoked pronounced temporal increases in IL-13 accumulation, which were largely ablated by pretreating the cocubated ASM cells with an anti-MHC class II blocking mAb. IL-13 protein levels were measured by ELISA. Data represent mean $\pm$ SE values (* = $p < 0.05$; ** = $p < 0.01$).

Role of IL-13 in mediating altered ASM responsiveness in the SEA-conditioned state

Given the above observations, we next investigated whether the induced release of IL-13 is responsible for producing the changes in agonist responsiveness observed in naive rabbit ASM tissues exposed to the SEA-conditioned medium (see Fig. 4). Accordingly, in comparable studies, agonist-mediated constrictor and relaxation responses were generated in rabbit ASM tissues exposed for 24 h to the SEA-conditioned medium collected on day 5 of cocubation of CD4⁺ T cells with SEA-presenting ASM cells, both in the absence and presence of pretreatment of the ASM tissues with an IL-13 neutralizing mAb. As depicted in Fig. 7, relative to control (vehicle-treated) tissues, ASM segments exposed to the SEA-conditioned medium exhibited significantly increased Tmax responses to ACh ($p < 0.05$) that averaged 110.2 ± 9.7 g/g ASM wt. vs the mean Tmax value of 95.1 ± 7.8 g/g ASM wt. obtained in the control tissues (Fig. 7A). Correspondingly, the ASM segments treated with the SEA-conditioned medium displayed significantly decreased Rmax responses to isoproterenol ($p < 0.01$) that averaged $31.0 \pm 5.1\%$ vs the mean Rmax value of $47.1 \pm 8.0\%$ obtained in the control tissues (Fig. 7B). As further shown, the induced changes in constrictor and relaxation responsiveness were completely abrogated in ASM tissues that were treated for 30 min with the IL-13 neutralizing mAb (1 μ g/ml) before exposing the tissues to the SEA-conditioned medium, providing Tmax and Rmax responses that were similar to those generated in control ASM segments. Contrasting with this observation, pretreatment with the IL-13 neutralizing mAb had no effect

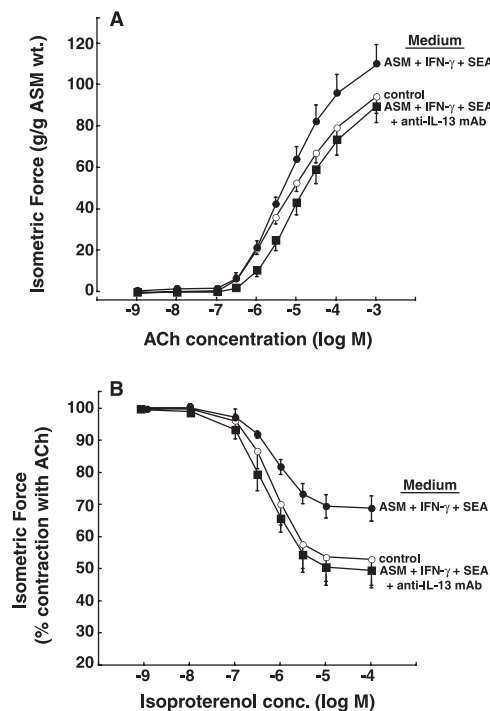


FIGURE 7. Role of IL-13 in mediating proasthmatic-like changes in ASM tissue responsiveness induced by conditioned medium from T cells coincubated with SEA-presenting ASM cells. **(A)** Comparison of constrictor dose-response relationships to ACh obtained in isolated naive rabbit ASM tissue segments exposed for 24 h to medium from control (vehicle-treated) ASM cell/T cell cocultures (*open circles*) and cocultures of T cells with IFN- γ -treated plus SEA-pulsed ASM cells both in the absence (*filled circles*) and presence (*filled squares*) of pretreatment of the ASM tissues with an IL-13 neutralizing mAb. **(B)** Comparison of relaxation dose-response relationships to isoproterenol generated in the same ASM tissue segments following their half-maximal contraction with ACh. Relative to their respective controls, the heightened T_{max} responses to ACh and impaired R_{max} responses to isoproterenol obtained in tissues exposed to the conditioned medium from cocultures containing IFN- γ -treated plus SEA-pulsed ASM cells were prevented by pretreating the tissues with the IL-13 neutralizing mAb. Data represent mean $\pm$ SE values from five paired experiments.

on either constrictor or relaxant responsiveness in control ASM tissues (data not shown).

Discussion

Microbial products can initiate a variety of chronic inflammatory disorders either via their potential to ligate pattern recognition receptors on immune cells or by serving as SAGs that specifically access adaptive immunity. Unlike classical peptide Ags that are processed and recognized in the context of self MHC class II molecules by a specific cognate TCR structure, SAGs do not undergo Ag processing and potentially elicit T cell activation by binding directly to invariant regions of MHC class II molecules on APCs and by coligating to the V β region of the TCR (5). As in a variety of other inflammatory conditions, SAGs have been implicated in the development of inflammatory airway disease (10), including in triggering T cell activation in severe asthmatic individuals (9). Moreover, it has been demonstrated that airway exposure to SAGs can elicit T cell-dependent airway inflammation and increased airway responsiveness in a murine model of nonallergic asthma (16). Despite this earlier evidence, the role of SAG exposure in the pathogenesis of asthma has not been adequately investigated, and little is known about how SAGs can initiate changes in airway

function. In light of this paucity of information, and given that ASM was previously shown to express MHC class II molecules (21, 25), the present study systematically examined the role and mechanism of action of SAg exposure on ASM function. Our results provide new evidence demonstrating that: 1) ASM cells can present the SAg, SEA, via their MHC class II molecules to resting CD4⁺ T cells, thereby eliciting T cell activation; and 2) this MHC class II-mediated ASM/T cell intercellular signaling evokes release of the potent Th2-type cytokine, IL-13, that, in turn, produces proasthmatic-like changes in ASM constrictor and relaxant responsiveness. Taken together, these findings are the first to implicate an innate role for ASM in potentially mediating the reported association between microbial SAg exposure, T cell activation, and severe asthma (9).

Ag presentation to both autologous and allogenic T cells by nonprofessional APCs has been clearly demonstrated for various parenchymal cell types including endothelial cells, epithelial cells, fibroblasts, and keratinocytes (1–3). As in these non-bone marrow-derived resident cell types, cell surface expression of MHC class II molecules has also been detected in vascular smooth muscle cells (31–33) and in ASM cells (21, 25), raising the possibility that smooth muscle cells also have the capacity to act as accessory APCs. Indeed, it has been demonstrated that Ag-pulsed vascular smooth muscle cells can stimulate CD4⁺ T cells, as evidenced by induced up-regulated T cell expression of CD25 and release of IL-2 (33). Our present observations extend these previous findings by demonstrating that SEA-pulsed ASM cells can stimulate resting CD4⁺ T cells by inducing up-regulated T cell expression of CD69 and CD25, and down-regulated T cell surface expression of CD4 (Fig. 2), all characteristic features of T cell activation. Interestingly, notwithstanding this evidence of an accessory APC role for MHC class II-bearing smooth muscle cells, and unlike other non-professional APCs, Ag-pulsed vascular smooth muscle cells and ASM cells were found to prevent proliferation of activated T cells through secretion of a putative mitogenic inhibitory factor (33, 34). Notably, under these circumstances, although T cell proliferation is blocked, this inhibitory effect was found to occur in the activated G₁ phase of the T cell cycle, which would allow for any ongoing synthesis and secretion of proinflammatory products (e.g., cytokines) by the activated T cells (33), as considered below.

We previously reported that ASM cells and CD4⁺ T cells in coculture exhibit bi-directional stimulatory cross-talk when either cell type is initially activated before coculture (21). Moreover, this reciprocal coactivation was attributed to the induced up-regulated cell surface expression and coligation of counterreceptor cellular adhesion molecules (i.e., ICAM-1 and LFA-1) and costimulatory molecules (i.e., CD40, CD40L, CD80 and CD86) correspondingly expressed by these cells (21). Given that this cooperative ASM/T cell intracellular signaling was further shown to mediate the induction of proasthmatic-like changes in ASM responsiveness (21, 22), the present study examined whether the conditioned medium from T cells stimulated by SEA-presenting ASM cells exerts a modulatory effect on ASM responsiveness. The results demonstrated that naive rabbit ASM tissues exposed to the SEA-conditioned medium exhibited heightened cholinergic agonist-mediated constrictor responsiveness and impaired β -adrenoceptor-mediated relaxation, both characteristic features of the perturbed ASM function in asthma (35); and these proasthmatic changes in ASM responsiveness were prevented by pretreating the cocultured SEA-pulsed ASM cells with an anti-MHC class II blocking Ab (Fig. 4). Thus, in accordance with previous evidence of direct cross-talk between ASM cells and CD4⁺ cells in coculture (21, 23), the present observations provide new information demonstrating that proasthmatic ASM/T cell intracellular signaling can be elicited in

response to MHC class II-mediated SAg presentation by ASM cells. In this regard, it is noteworthy that this SAg-induced effect was found to be associated with clustering of MHC class II and CD3 molecules at the ASM/T cell interface (Fig. 3), a finding that, to our knowledge, is the first to demonstrate the formation of immunological synapses between stimulated smooth muscle cells and CD4⁺ T cells. The ability of CD4⁺ T cells to form specific immunological synapses with ASM suggests that the ASM itself has the potential to function as a vital immune-regulatory organ that can directly access cells of the adaptive immune system, independent of any concomitant contribution from professional APCs.

The Th2-type cytokine, IL-13, has been identified as the primary CD4⁺ T cell-derived factor responsible for mediating allergen-induced airway hyperresponsiveness (28, 29). Given this evidence, together with that demonstrating that ASM cells can be induced to express IL-13 (30), and that IL-13 administration directly provokes changes in ASM constrictor and relaxation responsiveness (30, 36), we addressed the possibility that the SEA-presenting state is accompanied by altered expression of IL-13, relative to potential changes in expression of the Th1- cytokine, IFN- γ , in both the CD4⁺ T cells and cocultured ASM cells. The results demonstrated that coculture of T cells with SEA-presenting ASM cells evoked initial (at 6 h) up-regulated IFN- γ mRNA expression in both cell types and, by 24 h, distinctly up-regulated IL-13 transcripts were detected (Fig. 5A). Interestingly, at the latter time of cocultivation, IFN- γ mRNA was undetectable in the T cells, whereas the ASM cells exhibited concomitantly up-regulated expression of both the IL-13 and IFN- γ transcripts. Our finding that the stimulated CD4⁺ T cells underwent a temporal transition from initial Th1-type to subsequent Th2-type cytokine expression is consistent with recent reports demonstrating that human dendritic cells conditioned with the superantigen, SEB, are able to drive the polarization of naive allogenic T cells into the Th2 phenotype of cytokine expression (37). Additionally, PBMCs isolated from patients with atopic disease were found to respond to SEB by secreting a Th2-dominated cytokine pattern (13–15). Comparably, the present finding of induced coexpression of IL-13 and IFN- γ transcripts in the cocultured ASM cells is consistent with earlier evidence demonstrating that these cells are “nonpolarized” in their expression of both Th1- and Th2-type cytokines when sensitized with high IgE-containing atopic asthmatic serum (38). Regarding these observations, it is important to note that we found no evidence of induced cytokine expression in ASM cells that were either pretreated for 3 days with IFN- γ alone or subsequently also pulsed for 24 h with SEA before their inoculation with T cells (i.e., see 0 h in Fig. 5). In effect, this finding implies that, despite their up-regulated expression of MHC class II molecules in the IFN- γ -pretreated state, the ASM cells did not directly respond to SEA exposure and, hence, that their induced cytokine expression, which was detected only following coculture with T cells, occurred secondary to reciprocal (i.e., feedback) stimulation by the activated T cells.

In examining our results pertaining to the induced release of IL-13 protein into the cell culture medium under different experimental conditions, certain considerations are raised. Firstly, it should be noted that moderate but significant increases in IL-13 release were detected following direct treatment of T cells with IFN- γ plus SEA in the absence of cocultured ASM cells (Fig. 6). This observation is likely explained in light of previous reports demonstrating that SAgS can directly elicit T cell activation by binding to the V β -chain of the TCR and/or by coligating MHC class II molecules on one T cell with the TCR on another cell (39, 40). Furthermore, when cocultured with ASM cells pretreated under different conditions, the results demonstrated that: 1) despite

evidence of induced intercellular adhesion when exposing T cells to ASM cells treated with IFN- γ alone (Fig. 3A), this condition was not accompanied by the induction of IL-13 release; 2) release of IL-13 was significantly increased following coculture of T cells with SEA-pulsed ASM cell; and 3) this effect was strikingly enhanced when T cells were cocultured with SEA-pulsed ASM cells that were pretreated with IFN- γ (Fig. 6). Insofar as the markedly enhanced release of IL-13 was inhibited by pretreating the SEA-pulsed ASM cells with an anti-MHC class II blocking mAb, these data are consistent with the notion that the potentiated release of IL-13 was attributed to augmented MHC class II-mediated presentation of the superantigen by the IFN- γ -pretreated ASM cells. Finally, in concert with the observed induction of IL-13 release, our extended results demonstrated that the proasthmatic-like changes in constrictor and relaxation responsiveness exhibited by naive rabbit ASM tissues exposed to the SEA-conditioned medium were largely prevented by pretreating the tissues with an IL-13 neutralizing mAb (Fig. 7). The latter observation concurs with the known modulatory effects of IL-13 on ASM constrictor and relaxation responsiveness (30, 36), and indicates that the induced changes in ASM responsiveness were attributed to the presence of IL-13 in the SEA-conditioned medium. In this regard, in light of our observations demonstrating that both the cocultured T cells and SEA-presenting ASM cells exhibited induced IL-13 mRNA expression (Fig. 4), together with earlier evidence demonstrating that stimulated ASM cells can release IL-13 (30), the primary cellular source of the IL-13 protein detected in the SEA-conditioned medium remains to be identified.

In evaluating the overall significance of the present observations, it is relevant to note that, in contrast to IgE-mediated allergic asthma, nonallergic (“intrinsic”) asthma is allergen-independent and is known to be triggered by various factors including airway exposure to bacterial and viral pathogens. Indeed, microbial SAg exposure has been previously implicated in the pathogenesis of severe asthma, wherein both CD4⁺ and CD8⁺ T cells in the bronchoalveolar lavage (BAL) fluid from patients with poorly controlled asthma were found to express elevated levels of the TCR V β subtype, V β 8 (9). Moreover, contrasting with this finding in BAL fluid, enhanced V β 8⁺ T cell expression was not detected in the peripheral blood, indicating that these patients exhibited local T cell activation in the airways in response to SAg exposure (9). When considered in the light of this earlier evidence, our present observations support the notion that SAg presentation by ASM may, at least in part, account for the localized T cell activation associated with airway SAg exposure in severe asthma. This possibility remains to be systematically investigated, including within the broader context of the potential roles of other MHC class II-bearing resident airway cells (e.g., epithelial cells) in the pathogenesis of nonallergic asthma.

In conclusion, the present study investigated both the potential role of ASM as an accessory APC and its response to presentation of the superantigen, SEA, to CD4⁺ T cells. The results provided new evidence demonstrating that: 1) IFN- γ -pretreated human ASM cells exhibiting up-regulated cell surface expression of MHC class II molecules can effectively present SEA to resting CD4⁺ T cells and, accordingly, evoke T cell activation; 2) this phenomenon is associated with the formation of immunological synapses at the interface between the cocultured T cells and SEA-presenting ASM cells; 3) the conditioned medium from T cells cocultured with SEA-presenting ASM cells elicits proasthmatic-like changes in agonist-mediated constrictor and relaxant responsiveness in naive rabbit ASM tissues; 4) the CD4⁺ T cells exposed to SEA-presenting ASM cells exhibit initial transient up-regulated expression of IFN- γ mRNA followed by induced expression of IL-13

transcripts, whereas the cocultured ASM cells display concomitantly up-regulated expression of both IFN- γ and IL-13 mRNAs; 5) incubation of T cells with SEA-presenting ASM cells evokes a pronounced temporal increase in IL-13 protein release into the coculture medium; and 6) this MHC class II-dependent induction of IL-13 release by SEA-presenting ASM cells is responsible for producing the proasthmatic changes in ASM tissue responsiveness. Collectively, these observations support the novel concept that, apart from its inherent function as a regulator of airway tone, ASM may also play an important innate role as an accessory APC that presents superantigen to CD4⁺ T cells and, thereby, initiates Th2 cytokine-driven proasthmatic changes in ASM responsiveness. In this respect, the present findings are the first to identify a regulatory role for ASM that potentially contributes to the pathogenesis of intrinsic asthma, and that may mediate, at least in part, the reported interplay between SAg exposure, T cell activation, and severe asthma.

Disclosures

The authors have no financial conflict of interest.

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