522.21

Helper T Cells Potentiate the Effector Function and Growth of Anti-Tumor Cytotoxic T Lymphocytes

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The survival and proliferation of cytotoxic T lymphocytes (CTL) during the effector phase of the immune response is critical for the elimination of infectious agents and tumor cells. Our results show that the expansion and cytolytic function of CTL can be regulated by helper T lymphocytes (HTL) that provide strong costimulatory signals. The presence of HTL during the effector phase of anti-tumor (melanoma) CTL responses resulted in a significant increase in the proliferation potential of the CTL and in the enhancement of their overall cytolytic activity. The costimulatory activity of HTL required direct contact with CTL suggesting that cell surface interactions take place between these cells and that lymphokines alone are not sufficient to induce CTL proliferation during antigen stimulation. Because cross-linking of major histocompatibility complex (MHC) class II molecules on CTL results in their enhanced activation and proliferation, HTL may be able to potentiate CTL responses in an antigen-specific fashion.

522.22

Pivotal Role of Phosphoinositide-3 Kinase in Regulation of Cytotoxicity in Natural Killer Cells

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The MAPK-ERK plays a critical role in natural killer (NK) cell lysis of tumor cells, but its upstream effectors are unknown. We now show that inhibition of phosphoinositide-3 kinase (PI3K) in NK cells blocks PAK1, MEK, and ERK activation by target cell ligation, interferes with perforin-granzyme B movement toward target cells, and suppresses NK cytotoxicity. Dominant-negative N17Rac1 and PAK1 mimic the suppressive effects of PI3K inhibitors, whereas constitutively-active V12Rac1 has opposite effects. V12Rac1 restores the activity of downstream effectors and lytic function in LY294002- or wortmannin-treated but not PD98059-treated NK cells. These results document a specific PI3K [ratr] Rac1 [ratr] PAK1 [ratr] MEK [ratr] ERK pathway in NK cells to effect lysis.

MECHANISMS OF AND INTERVENTIONS FOR ASTHMA AND ALLERGIC DISEASES (523.1-523.4)

523.1

The production of species-specific monoclonal antibodies (Mabs) against the allergenic and toxigenic fungus *Stachybotrys chartarum*

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Fungi in occupational and domestic indoor environments have been associated with adverse allergenic, infectious and/or toxic health effects. Among the species of much recent attention has been *Stachybotrys chartarum* which has been shown to produce a variety of mycotoxins which may contribute to pulmonary inflammation, hemorrhage and immune suppression. In order to better understand the importance of *S. chartarum* in indoor environments, accurate and precise monitoring methods are required and in this study we report the cross-reactivity patterns of Mabs which can be used for exposure measurements.

Five IgM (3B2, 1D4, 10A5, 9F9 and 4E12) and one IgG-secreting cell line (9B4) were raised in mice against spores of *Stachybotrys chartarum* and tested for cross-reactivity against mycelia or spores of 59 fungal isolates representing 40 species commonly found in indoor environments including species of *Alternaria, Aspergillus, Aureobasidium, Cladosporium, Epicoccum, Eurotium, Memnoniella, Myrothecium, Penicillium, Stachybotrys, Trichoderma, Ulocladium* and *Wallemia*. All Mabs except 9B4 were found to cross-react with other spores or mycelia of multiple species. Mabs 3B2 and 10A5 reacted with several species of *Cladosporium, Stachybotrys, Memnoniella, Myrothecium* and *Aureobasidium*. Mabs 9F9 and 4E12 showed a similar pattern but at a much lower quantitative level and did not react with *Myrothecium*. Mab 1D4 reacted with multiple species of *Stachybotrys, Memnoniella* and *Trichoderma*. Mab 9B4 was the only Mab found to be species-specific for *Stachybotrys chartarum* and reacted only with spores but not with mycelium. Heat treatment and periodate oxidation indicate that Mab 9B4 recognizes an epitope expressed by a protein and that all other Mabs bind to secreted carbohydrates.

The results show that cross-reactivity is widespread among fungi and that spores and mycelium may express unique epitopes.

523.2

REGULATION OF IL-13 BY HISTAMINE IN TH2 CELLS

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Histamine affects the balance of Th1 (T helper type 1) and Th2 (T helper type 2) cytokines by shifting cytokine production from a Th1 to a Th2 pattern. IL-13 (interleukin-13) is an important autacoid mediator that has been implicated in the development of allergic disease. This study was designed to investigate the mechanisms of regulation of IL-13 by histamine in Th2 cells. D10.G4.1 cells, a murine Th2 cell line, were treated with histamine (10^{-4}-10^{-6}M) and then activated with PMA (phorbol 12 myristate 13-acetate) plus ionomycin or αCD3. Levels of IL-13 production were then measured by ELISA (enzyme-linked immunosorbent assay) and semiquantitative RT-PCR (reverse transcriptase-polymerase chain reaction). Cells were pretreated with histamine receptor antagonists pyrilamine, ranitidine, cimetidine and thioperamide to determine the involvement of histamine receptors. Cells were also pretreated with PKA (protein kinase A) inhibitors H-8 (N-[2-(methylaminoethyl)]-5-isoquinoline-sulfonamide) and *Rp*-cAMPS (*Rp*-diastereomer of adenosine cyclic 3'5'-phosphorothioate) and Jak-STAT (janus kinase - signal transducer and activator of transcription) inhibitor tyrphostin AG490 prior to the addition of histamine. H-8 is an inhibitor of the catalytic subunit of PKA while *Rp*-cAMPS is an inhibitor of the regulatory subunit of PKA. Tyrphostin is an inhibitor of Jak2, Jak3, STAT1, STAT3 and STAT5. Finally, cells were pretreated with IL-12, a cytokine known to repress STAT6 DNA binding. We found that histamine dose-dependently enhanced IL-13 secretion and transcripts in Th2 cells via H1 and H2 receptors. Pretreatment of cells with H-8 and *Rp*-cAMPS prevented histamine-induced secretion and transcription of IL-13. Tyrphostin partially reversed stimulation of IL-13 secretion and transcription by histamine. Likewise, pretreatment of Th2 cells with IL-12 also reversed histamine's effects on IL-13 secretion from stimulatory to inhibitory. These observations suggest a role for PKA and the Jak/STAT pathway in histamine-mediated elevation of IL-13 secretion and transcription.

523.3

Elevation Of Intracellular Ca^{2+} In Human Eosinophils Upon Activation As Assessed By Flow Cytometry.

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We have measured calcium ionophore (CI) induced Ca^{2+} flux signals in human eosinophils using FLUO 3/AM as the Ca^{2+} indicator dye. The eosinophils, isolated from patients with eosinophilia using Percoll gradients, were loaded with the FLUO 3/AM dye at 37°C water incubator. Loaded cells were maintained at 37°C, and activated with CI. Flow cytometry was performed by gating on the granulocyte population, and freshly separated neutrophils were tested for comparison. Calcium flux is expressed as mean geometric mean and mean peak channel±SE.

	Geometric Mean	Peak Channel
Neutrophils	4.3±0.7 (4)	3.8±0.6 (4)
Neut+CI(1min)	41.3±8.4 (3)	43.1±7.5 (3)
Neut+CI(15min)	31.1±9.2 (3)	35.3±9.5 (3)
Eosinophils	2.8±1.2 (2)	1±0 (2)
Eos+CI(Peak I/1min)	30.1±1.0 (2)	9.5±1.5 (2)
Eos+CI(Peak II/1min)	36.3±2.7 (2)	33±0 (2)
Eos+CI(15min)	21.8±2.3 (2)	29.0±10.0 (2)

Activating neutrophils with CI resulted in 10-fold increase in Ca^{2+} flux and shift in peak channel by 1 minute. Eosinophil activation at 1 minute resulted in 2 distinct peaks (at approximately channel 10 and 33), indicating there may be subpopulations. Eosinophils cultured with IL-5 (data not shown) also had 2 peaks with a shift-in-peak-2-to-a channel 41. Analysis 15 minutes post activation of the eosinophils resulted in the merging of the two peaks. Thus, assessment of intracellular calcium may be a useful way for studying eosinophil activation.

523.4

Contrasting Effects Of (interleukin (IL)-5 And Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) On Human Eosinophils *In Vitro*.

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Interleukin (IL)-5 and GM-CSF have both been reported to stimulate eosinophil function and viability *in vitro*. We tested these cytokines using a method we devised for culturing eosinophils with autologous mononuclear cells (MNC). Heparinized blood was obtained from patients with various disorders associated with eosinophilia and from normal newborns (cord blood). The patient blood was then layered over 75% (72.5% for cord) layer of Percoll and spun down, resulting in separation of the erythrocytes (bottom) from the leukocytes. The leukocytes were then suspended in RPMI media with 20% fetal calf serum at 10^6cells/ml, and placed in a 37°C, CO_2 incubator. Differential cell counts were done at weekly intervals to determine the percentage of eosinophils still viable. Flow cytometry was also done weekly to determine the number of eosinophil activation. By one week, most of the neutrophils had disappeared, leaving eosinophils as the predominant granulocyte population. In the absence of any cytokines, the eosinophils also declined. The percentages of eosinophils increased when IL-5 was present using blood from both patients and newborns. GM-CSF 100 ng/ml resulted in an increase in eosinophils at week 1 using patient cells, but then rapidly declined by week 2. Neonatal eosinophils were not enhanced by GM-CSF. The addition of GM-CSF to cultures stimulated with IL-5 resulted in inhibition of IL-5 enhancement of eosinophil survival. Flow cytometry studies also indicated that GM-CSF has an inhibitory effect on eosinophil activation as assessed by CD69 expression. This inhibition by GM-CSF may be due to other cytokines released by monocytes, which are also stimulated by GM-CSF in this culture system. These studies may have implications for long-term use of GM-CSF in clinical trials.

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Experimental Biology 2001®
Orlando, Florida
March 31-April 4, 2001

ABSTRACTS
PART I

Abstracts 2.1–537.42

Official Publication of the Federation of American Societies for Experimental Biology
Volume 15, Number 4, March 7, 2001