行政院所屬各機關出國報告

（出國類別：進修　　）

美國約翰霍普金斯大學
氣喘過敏中心進修報告

服務機關:台中榮民總醫院
出國人　職　　稱: 主治醫師
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出國地區: 美　　國
出國期間: 91年9月15日至92年9月14日
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行政院輔導會臺中榮民總醫院

聯絡人／電話:

王天禔／04-23592525-2024

出國人員:

陳怡行　行政院輔導會臺中榮民總醫院　內科部免疫風濕科　主治醫師

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分類號/目: J2／西醫　／

關鍵詞:　過敏,氣喘,減敏治療,DNA疫苗

內容摘要: 職於民國91年9月至民國92年9月至美國約翰霍普金斯大學氣喘過敏中心進修過敏免疫學. 進修期間,除於臨床觀察各式過敏性疾病的診療技巧以外, 亦加入由指導老師Creticos教授主持, 由美國國家衛生院贊助之過敏病 DNA疫苗研究計劃. 在約大氣喘過敏中心進修的一年中, 職每週兩天跟隨Creticos教授門診,其餘時間則於Schroeder教授實驗室中,從事有關 DNA疫苗於人體作用機轉相關的基礎免疫研究. 進修一年期間, 預定學習之項目及研究工作均有豐碩之果.以第一作者身份已完成論文三篇. 期望返國之後,能將約翰霍普金斯大學氣喘過敏中心的成功經驗, 帶回本院, 使本院過敏學科的發展,能更上一層樓.

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陳怡行/台中榮民總醫院/內科部過敏免疫風濕科/主治醫師 /04-23592525 轉 3333

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摘要

職於民國 91 年 9 月至民國 92 年 9 月至美國約翰霍普金斯大學氣喘過敏中心進修過敏免疫學. 進修期間,除於臨床觀察各式過敏性疾病的診療技巧以外, 亦加入由指導老師 Creticos 教授主持, 由美國國家衛生院贊助之過敏病 DNA 疫苗研究計劃. 在約大氣喘過敏中心進修的一年中, 職每週兩天跟隨 Creticos 教授門診,其餘時間則於 Schroeder 教授實驗室中,從事有關 DNA 疫苗於人體作用機轉相關的基礎免疫研究. 進修一年期間,預定學習之項目及研究工作均有豐碩之果.以第一作者身份已完成論文三篇. 期望返國之後,能將約翰霍普金斯大學氣喘過敏中心的成功經驗, 帶回本院, 使本院過敏學科的發展,能更上一層樓.

目的

隨著台灣社會的進步與生活習慣逐漸的西化的改變，過敏病已經成爲台灣增加最快速的慢性病. 根據統計，由民國 63 年至民國 83 年二十年間, 台北市學童的氣喘病盛行率增加 8.3 倍,達到 10.79%, 而最近衛生署包含成人的流行病學調查, 台灣大約有百分之二十的人有過敏性鼻炎，百分之七左右的人有過敏性氣喘. 在可預見的未來,過敏病將成爲台灣最普遍的慢性病之一, 且與高血壓,糖尿病以中老年人爲多不同的是,過敏病多半影響兒童與青壯年,衝擊的是國家未來及現在的主人翁, 如何更有效地治療及預防過敏病,將成爲一個極重要的課題.

本院過敏免疫風濕科過去於每週三下午設有「過敏病特別門診」服務過敏病患者.自民國 87 年起, 爲因應過敏病患者逐年之增加,遂將原過敏病特別門診每週再增加一診, 移至每週二、五上午,並更名爲「過敏氣喘特別門診」.至民國 91 年八月底近四年來,已服務病患超過 2 萬人次,且仍持續增加中.

鑑於本院過敏病之業務成長之快速, 而近年來國外過敏病之研究突飛猛進, 新的診斷治療方法日新月異, 遂有出國進修過敏病診療之需求.

過程

職於民國 91 年 9 月至民國 92 年 9 月至美國約翰霍普金斯大學氣喘過敏中心進修過敏免疫學. 美國美國約翰霍普金斯大學醫學院夙有盛名, 近年已連續 13 年獲得美國 U.S. News 雜誌評鑑,爲全美最佳醫院之第一名, 甚至領先哈佛大學醫學院之麻州綜合醫院及著名的 Mayo 診所. 而其過敏免疫科之氣喘過敏中心, 亦已多年於相關評鑑中,與丹佛之國家猶太醫院並列爲全美最佳的過敏病中心, 遂擇定此校爲進修之地點.

約翰霍普金斯大學氣喘過敏中心爲約翰霍普金斯醫院內科部過敏免疫科整合胸腔內科,耳鼻喉科及皮膚科從事過敏病研究者所成立之過敏病研究中心,位於美國馬里蘭州巴爾的摩市東約翰霍普金斯大學的港景校區(Bayview Campus). 約翰霍普金斯大學於 1923 年便於內科部內設有過敏之 program, 但直至 1970 年才由 Laurance M. Litchenstein 教授及 Philip S. Norman 教授共同創立獨立的過敏免疫科. 1989 年整合各相關科, 遷移至目前的港景校區新大樓, 即目前之氣喘過敏中心. 目前共有各級教授 42 人,研究員及訪問科學家 25 人.

職跟隨之指導教授,爲約大過敏免疫科之臨牀主任 Peter S. Creticos 教授. Creticos 教授的專長,是呼吸道的過敏病及減敏治療. 減敏治療研究的一些重要論文,如減敏治療中免疫球蛋白之變化, 減敏疫苗最佳維持劑量的關念, 胜肽減敏治療等,均是出於其研究團隊之手. Creticos 教授近年最重要之研究, 是將具免疫刺激效果之 CpG 寡聚核苷酸與豬草花粉之主要過敏原 Amb a1 以共價鍵結合成 DNA 疫苗, 運用於人體上, 治療過敏病. Creticos 教授本身爲臨床醫師, 其研究有關基礎免疫的部份, 由其團隊中之 John T. Schroeder 教授負責. Schroeder 教授的專長,主要爲嗜鹼性細胞與組織胺的生理. 在約大氣喘過敏中心進修的一年中, 職每週兩天跟隨 Creticos 教授門診, 學習各種過敏病的臨床診療,其餘時間則於 Schroeder 教授實驗室中,從事有關 DNA 疫苗於人體作用機轉相關的基礎免疫研究.

進修期間, 職於 Schroeder 教授實驗室主要之研究主題, 爲 CpG 寡聚核苷酸之受體 Toll-like receptor 9 在漿樣樹突細胞(plasmacytoid dendritic cells) 及嗜鹼性細胞之表現及調控, 以及漿樣樹突細胞受 CpG 寡聚核苷酸刺激後,所泌之主要細胞激素,甲型干擾素, 對嗜鹼性細胞之 IgE 媒介反應及非 IgE 媒介反應之調控.

臨床部份, 除跟隨 Creticos 教授學習臨床技巧以外, 約大氣喘過敏中心有完整

之過敏原誘發試驗實驗室，對診斷罕見的過敏疾病，及設計過敏病相關研究,是一極重要的工具，也是職此次進修觀摩的重點.

職於進修期間，亦曾多次隨 Creticos 教授至美國國家衛生院參加 DNA 疫苗計畫之研究會議，觀察其研究團對如何將實驗室的研究,與臨床的數據,運用各種統計方式,做一整合，並協助 Creticos 教授做部份數據之分析，其經驗可做爲將來返國從事研究工作之經驗參考.

職於進修一年期間,預定學習之項目及研究工作均有豐碩之果.以第一作者身份已完成論文三篇，分別爲原始論文" Interferon-α inhibits IL-3 priming of human basophil cytokine secretion but not leukotrienes and histamine release" (附件一)及" Expression of toll-like receptor 9 in human basophils: a potential cell target for CpG-DNA" (附件二)，與 Creticos 教授合著之綜合評論 "New approaches of immunotherapy: allergen vaccination with immunostimulatory DNA" (附件三). 另 DNA 疫苗臨床成果之系列論文,亦承 Creticos 教授允諾列爲共同作者.

心得

於約翰霍普金斯大學氣喘過敏中心一年的心得,可從幾個方面來說:

(一) 臨床 :

- 軟體方面: 過敏病的診察,除了傳統內科學的詳細病史詢問及理學檢查以外, 對患者的生活環境及工作環境可能暴露的過敏原,亦必須有詳盡的了解, 所以比起其他學門, 更需走入人群,以了解各種生活型態., 即使是知名教授, 看診時亦是不厭其煩詢問,相當值得學習. 約大氣喘過敏中心病患就診前, 需事先填妥一詳細的起居用藥細節問卷,(如附件四) 不僅可以使看診時間更有效率, 也不易產生疏漏, 極適合本科參考.
- 過敏原: 過敏原有極強之地域特性. 在約大, 可見全美不同地區來的患者, 便有不同的過敏原.台灣也極需有更多的本土的過敏原研究, 以使我們臨床使用的過敏原試劑及減敏疫苗更接近本地患者的需求. 約大對過敏原的皮膚試驗, 除例行採用目前廣泛使用(本科目前亦用此法)的針刺法(skin prick test)以外, 亦保留傳統的皮內注射法, 供特殊病患使用,以增加檢查的敏感度.(如附件五至九)
- 減敏疫苗: 雖然近年歐洲有關減敏疫苗的研究,較傾向於不混合多種過敏原, 注射時將各單一過敏原注射在不同部位,以免某些過敏原中的酵素活性,改變了其他相混過敏原表位(epitope)的活性. 約大對多重過敏者仍採用混合疫苗方式,但是將酵素活性雷同的過敏原配在同一小瓶,如此可免這些多重過敏患者於減敏注射時,要挨好幾針. 本科目前是採用歐洲的方式, 不混合過敏原, 將來是否採行混合疫苗方式, 尚須評估. (如附件十)
- 診間動線設計: 過敏氣喘中心爲一馬蹄形建築, 所有因過敏氣喘疾病來就診的患者,都統一在樓層中央的掛號處報到. 環繞掛號處四周的, 依序爲後診區, 衛教室, 診間, 抽血區及過敏原皮膚試驗區, 肺功能室及誘發試驗室, 及檢敏治療室. 所以患者只需在同一建物同一樓層,即可完成所需的所有程序, 免於在醫院各建築間奔波之苦, 爲非常人性化及有效率之設計.

(二) 研究: 約大氣喘過敏中心 42 位各級教授中, 幾乎每個人皆有不同的專長. 從事臨床工作的 8 位教授, 有專長藥物過敏者, 有專長減敏治療者,有專長過敏性鼻炎極氣喘者, 有專長慢性蕁麻疹者, 有專長異位性皮膚炎者, 有專長嗜

伊紅性疾病者，再加上其他從事基礎免疫研究者,幾乎過敏病所涵蓋的範疇,皆有人可司其職，難怪可成爲全美之重鎮. 在約大印象特別深刻的，是臨床教授與基礎研究教授間的緊密合作關係. 如職在美之兩位指導教授，一司臨床,一司基礎，既可顧及研究之深度，也不會脫離臨床實際面，其成果也隨時可由 bench 回到 bedside，運用於臨床，實爲一極佳的合作模式.

（三） 教學: 氣喘過敏中心沒有住院病床,所以不負責住院醫師的訓練,主要的受訓者，爲每屆兩名的臨床研究員,及爲數眾多的基礎研究員.(如附件十一)

- 研究員訓練：約大過敏免疫科每屆收兩名內科醫師背景之臨床研究員，與小兒免疫科之研究員聯合訓練. 其臨床研究員第一年每週於門診跟診四天，第二年每週跟診兩天，其餘時間必須從事實驗室研究工作，以求臨床與研究之平衡. 每年七月跟八月新研究員剛來時，有密集的orientation課程,(附件十二) 像新生訓練一樣,給新來的研究員對整個過敏免疫學領域有一個概念，由所有氣喘過敏中心的教授授課,之後則於每周三中午由研究員負責一個鐘頭的 case study 或 review.研究員的 talk 每次有不同教授負責提供諮詢，除剛開始的兩個月，其餘的訓練精神仍以主動學習爲主. 週三下午則有 research conference，由過敏免疫學各領域的專家演講其研究的內容. 週五中午的演講則多爲醫院內各科資深臨床醫師就其各專科與過敏病相關的部份做演講. 每月有一個週五下午爲期刊討論會. 比較特別的是，討論會的形式爲一個研究員與一個教授配對,就同一個主題,提出兩篇相關文章,除須於五分鐘內將期刊文章內容簡報完成以外,尚需提出批判,尤其是研究設計的部份，藉此來訓練閱讀期刊的技巧,而不是照單全收文章內容.
- 學生: 過敏氣喘中心在每年暑假及寒假,也接受對科學研究有興趣的高中生，大學生及醫學院學生到實驗室學習. 學習的內容,則視各教授實驗室研究主題不同,而有所差異.但基本上，整個環境相當鼓勵這些年輕人做科學研究嘗試. 即使是無經驗的高中生，教授們也是盡心與以指導，培育研究的種子，令人印象深刻.

建議

由目前台灣過敏兒越來越多的情況來看，過敏病在可預見的將來，將成爲內科學領域極重要的一個慢性病．本院過敏免疫風濕科在台灣一向享有盛名,多年來免疫風濕疾病的臨床診療，也一直居於全台領先位置，如何在保持現有成就外，繼續於內科過敏學領域精益求精，使本科過敏病的診療研究，亦能達到相同成績，實爲一任重而道遠之任務．

由約翰霍普金斯大學氣喘過敏中心的成功經驗，對應於本院目前的情況，職有以下建議:

(一) 過敏病門診區功能與動線,應可再做規劃，以增加利用效率及患者就診方便度．

(二) 本科應持續逐步建立純化本土過敏原之能力，以便將來可運用於臨床之診斷與檢敏治療．

(三) 本科應加強與基礎醫學研究學者之合作，方能由現有的臨床經驗爲根基，撞擊出更美麗有深度的研究．

(四) 本科應加強過敏病相關的教學，以啓發年輕醫師的興趣，鼓勵更多年輕醫師投入過敏病的領域．

Interferon-α Inhibits IL-3 Priming of Human Basophil Cytokine Secretion but not Leukotriene C4 and Histamine Release

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Abstract

Background: Innate immune responses play a critical role in determining the course of acquired immunity, including that associated with allergic disease. Type I interferons, which are generated early on in these reactions, are important soluble factors that prime for Th1-like activity.

Objective: Since human basophils secrete IL-4 and IL-13 in response to both IgE -dependent and -independent stimuli, we tested whether IFN-α, a major type I IFN, affects the production of these Th2 cytokines and/or mediator release from these cells.

Methods: Basophils isolated from blood were treated with IFN-α in the presence and absence of IL-3 priming before stimulating through the IgE receptor to release histamine, LTC4, and IL-4. Effects of IFN-α on IL-3-mediated IL-13 secretion and basophil survival were also tested. IFN-α receptor expression was determined by RT-PCR.

Results: IFN-α specifically inhibited the effects IL-3 has on basophil cytokine secretion. Enhanced secretion of IL-4 resulting from IL-3 priming was significantly inhibited in cells concurrently cultured with IFN-α. This effect was specific for cytokine generation, since histamine and LTC4 were unaffected. Furthermore, IFN-α blocked IL-13 secretion directly induced by IL-3. While IFN-β also possessed some inhibitory activity, IFN-γ (a type II IFN) had no effect on basophil cytokine secretion. Basophils constitutively expressed mRNA for the

type I IFN receptor and IFN-α did not affect basophil viability with regard to inhibition of cytokine secretion.

Conclusion: These results support the belief that early innate immune responses resulting in IFN-α production negatively regulate allergic responses by also inhibiting priming of basophil cytokine release.

Key words: IgE, innate immunity, hypersensitivity, cytokine priming, histamine, leukotriene

Abbreviations

IL: Interleukine

IFN: Interferon

LTC4: Leukotriene C4

IFN-αR: Interferon-α receptor

FBS: Fetal bovine serum

BSA: Bovine albumin

EDTA: Ethylenediamine tetraacetate acid

PIPES: Piperazine-N,N'-bis-[2-ethanesulfonic acid]

IMDM: Iscove's modified Dulbecco's medium

HPRT: Hypoxanthine phosphoribosyl transferase

PDC: Plasmacytoid dendritic cell

Introduction

Basophils are one of several cell types known to selectively infiltrate allergic lesions, both naturally and when experimentally induced [1-4]. The significance of these cells in allergic inflammation has increased during the last decade with evidence that they secrete large quantities of the pro-inflammatory cytokines, IL-4 and IL-13, in addition to releasing the potent inflammatory mediators, histamine and LTC4 [5]. Activation through the IgE receptor plays a critical role in the release of all these products. Furthermore, there are several cytokines that markedly enhance this response in what is often referred to as "priming". Of these priming cytokines, none appear to have greater activity than IL-3, which not only primes for IgE-mediated release, but is also known to directly induce the secretion of IL-13, particularly from basophils of allergic subjects [6-8]. In contrast to the priming qualities of IL-3, there are currently no reports of cytokines that suppress basophil mediator release and/or cytokine generation.

Interferon (IFN)-α is a major member of the type I IFN family [9]. While initially characterized for its potent anti-viral activity, there is increasing evidence that IFN-α also plays a significant role early on in the course of Th1-like immune responses. Among the more recent activities attributed to this cytokine is its ability to induce dendritic cell maturation, enhance the cytotoxic activity of macrophages and natural killer (NK) cells, support antibody production by B cells, and promote Th1-like responses by increasing the expression of IL-12 and IFN-γ [10].

Although the cell most responsible for IFN-α production has remained elusive for many years, there is mounting evidence that plasmacytoid pre-dendritic cells account for more of this cytokine than any other immune cell [11]. When stimulated with viral replication products, such as double-stranded-RNA (dsRNA) or unmethylated CpG motifs of bacterial DNA, these cells are capable of producing 200 to 1000 times more IFN-α than any other white blood cell [12]. These observations, in part, have led to the belief that IFN-α plays an essential role in linking innate and acquired immune responses [13].

As the first available therapeutic cytokine, IFN-α has also been widely used in the treatment of a variety of neoplastic conditions [14], chronic hepatitis B and C infection [15, 16], hypereosinophilic syndrome [17], systemic mastocytosis [18], and histiocytosis [19]. Moreover, there are recent reports of IFN-α being used in the treatment of steroid–resistant asthma and intractable atopic dermatitis with favorable results, even though no definitive mechanisms of action have been determined [20]. In accordance with these latter findings, Bufe et al. have shown a significantly lower amount of virus-induced IFN-α in patients with allergic asthma than in healthy children and patients with non-allergic asthma, suggesting that its decreased expression may account, in part, for these conditions [21].

In this study, we show evidence for the first time that type I interferons, specifically IFN-α, inhibit the effects IL-3 has on basophil cytokine secretion, suggesting one mechanism by which innate immune responses might negatively regulate allergic reactions.

Material and Methods

Special Reagents

The following reagents were purchased: crystallized human serum albumin (Calbiochem-Behring Corp, La Jolla, CA, USA); Piperazine-N,N'-bis-2-ethanesulfonic acid (PIPES), FBS, and crystallized BSA (Sigma Chemical Co, St Louis, MO, USA); gentamicin, Iscove's modified Dulbecco's medium (IMDM), non-essential amino acids (100X stock) and 0.4%Trypan blue (Life Technologies, Inc, Grand Island, NY, USA); Percoll (Pharmacia Biotec, Inc, Piscataway, NJ); recombinant human IL-3, interferon-α2, interferon-β and interferon-γ (Biosource, Inc. Camarillo, CA, USA). The polyclonal anti-human IgE antibody used in these experiments was made in a goat. [22]

Special Buffers and Media

10x PIPES buffer (250 mM PIPES, 1.10 M NaCl, 50 mM KCl, pH 7.3) was stored at 4°C as a stock solution. PIPES-albumin-glucose (PAG) was made by diluting 1 part 10x PIPES with 9 parts deionized water and contained 0.003% human serum albumin and 0.1% D-glucose. PAG-EDTA additionally contained 4 mM EDTA. Isotonic Percoll (referred to in this article as 100% Percoll) was prepared by mixing 1 part 10x PIPES and 9 parts Percoll. Working solutions of Percoll at 55% (density, 1.072 g/ml) and 61% (density, 1.081 g/ml) were made by

mixing the appropriate amounts of 100% Percoll with 1x PIPES. Conditioned medium (C-IMDM) consisted of IMDM supplemented with 5% heat-inactivated (56°C for 30 minutes) FBS, 1x nonessential amino acids, and 5μg/ml gentamicin.

Cell Purification and Culture

Basophils were purified from either fresh blood or from residual cells of normal donors undergoing leukopheresis. Venipuncture was performed on non-medicating consenting adults (age range, 21-55 years) by using guidelines approved by either the Western Institutional Review Board (Seattle, Washington) or by the Johns Hopkins University Institutional Review Board. Subjects were not selected based on their allergic status. Mixed-leukocyte suspensions containing basophils were prepared using double-Percoll density centrifugation, as previously described [23]. The basophils were additionally purified to 95-99.9% by negative selection using an antibody cocktail and microbead protocol (StemCell Technologies, Vancouver, Canada) followed by gravity filtration through a magnetized LS-column attached to a MidiMACS magnet (Miltenyi Corp., Auburn, CA, USA). The percentages of basophils were determined by counting Alcian blue positive and negative stained cells on a Spiers-Levy chamber [24]. Cells were cultured in 96-well flat-bottom microtiter plates (in duplicate) with C-IMDM and were warmed to 37°C, 5% CO_2 before adding reagents that were also equilibrated to these conditions. For the concurrent detection of histamine, LTC4, and IL-4 following IgE-mediated activation,

basophils were pre-incubated in C-IMDM alone or with the indicated concentrations of IL-3, interferons, or a combination of both. After 18 h incubation, the cells were then activated with anti-IgE antibody (final concentration of 10-25 ng/ml) and cultured for an additional 4h. In experiments investigating IL-3-dependent IL-13 secretion, interferon and IL-3 were added simultaneously and the cells cultured for 18-20 h, as previously described [7]. Portions of the cell-free supernatants were analyzed for histamine and LTC4 using automated fluorimetry and RIA, respectively, and according to protocols previously detailed [23, 25]. Cytokine protein was detected by ELISA using IL-4 (e-Bioscience, International,Camarillo, CA) and IL-13 (Immunotech, Westbrook, ME) commercial kits.

RNA isolation

Total RNA was isolated from 0.5-1.0x10^6 basophils using the RNAzol protocol (Tel-test Inc., Friendswood, TX, USA). Following isopropanol precipitation, the RNA was washed with 70% ethanol and nearly dried under vacuum. Subsequently, the RNA was reconstituted in diethylpyrocarbonate (DEPC)-treated water and stored at –70˚C.

Detection of IFN-α receptor (IFN-αR) mRNA by RT-PCR

Reverse transcription (RT) and polymerase chain reaction (PCR) were performed using the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, CT, USA), as described elsewhere

[26]. Both the forward primer sequence: 5'- AGTGTTATGTGGGCTTTGGATGGTTTAAGC-3' and the reverse primer sequence: 5'-TCTGGCTTTCACACAATATACAGTCAGTGG-3' for the IFN-αR_1 subunit (accession No. NM 000629) have been previously reported [27]. Resolution of the cDNA product (765 bp) after amplification was done in 3% agarose gels. Imaging was performed with an Electrophoresis Documentation and Analysis System 290 (Kodak Scientific Imaging Systems, New Haven, Conn.).

Basophil viability

Basophils were cultured in C-IMDM alone, with IL-3 (100 pg/ml) or IFN-α (100U/ml), and with a combination of these two cytokines. Basophil viability under these conditions was determined after 1, 24, 48, 72, 96, and 120 hours incubation using trypan-blue exclusion with enumeration of the cell counts made using a Spiers-Levy chamber.

Statistical analysis

Data are presented as mean± standard error of the mean (s.e.m.) unless otherwise indicated. Statistical analysis was performed with SPSS 10.0 software (SPSS, Inc, Chicago, IL, USA). Nonparametric tests (Wilcoxon signed rank test or Friedman test as indicated) were used to determine the statistical significance of differences. P values less than 0.05 were considered significant.

Results

The effects of IFN-α on IgE-mediated cytokine and mediator release by IL-3 primed human basophils

In the first series of experiments, we tested the effects of IFN-α on IgE-mediated cytokine and mediator release using various culture conditions that we routinely use in screening the effects of pharmacological and biological substances on basophil secretion. While short (e.g. 15 min) pre-incubations with IFN-α produced no identifiable effects on the IL-3 priming of mediator release and cytokine secretion (data not shown), this type I IFN did affect basophil function after 18 h pre-incubation. Figure 1a shows the effects of IFN-α on the IgE-mediated release of IL-4 in both the presence and absence of priming with low concentrations of IL-3 (100 pg/ml) for 18 h. Whereas increasing concentrations of IFN-α had no effect on the IL-4 secreted in response to anti-IgE alone (i.e. without IL-3 priming), the 4.5-fold increase in IL-4 achieved with IL-3 priming was significantly inhibited at concentrations of 500 and 5000 U/ml IFN-α. In contrast to this inhibitory effect of IFN-α on the priming of IgE-mediated IL-4 secretion, only a marginal, yet insignificant, inhibition of LTC4 was observed even with the highest (5000 u/ml) concentration tested (figure 1b). Moreover, IFN-α produced no effects on the histamine released in these cultures, either in the presence or absence of IL-3 priming (figure 1c).

Effect of IFN-α on IL-3-mediated IL-13 production

Since IFN-α precluded IL-3 priming of IgE-mediated IL-4 secretion, we decided to investigate whether this type I IFN would also affect other IL-3-dependent basophil responses. In particular, we, and others, have shown that basophils secrete large amounts of IL-13 when exposed to IL-3 alone [6, 28]. Therefore, we tested a wide range of IFN-α concentrations on this response, adding them simultaneously with several concentrations of IL-3 and culturing basophils for 18-20 h to measure IL-13 protein. As shown in figure 2, IFN-α inhibited IL-13 production from basophils in a dose-response fashion when activated with IL-3 at either 1 or 10 ng/ml. In fact, a significant 20-30% inhibition was observed with as low as 1 U/ml IFN-α, and nearly 60 % inhibition seen with 1000 U/ml. While significant inhibition by IFN-α was also seen when IL-13 was induced with 0.1 ng/ml IL-3, the much lower levels of IL-13 (20±9 pg/ml) secreted under these conditions likely account for the lack of discernible dose-response inhibition.

Effect of Type I vs. Type II interferons on IL-3-mediated IL-13 production

IFN-β represents another well-characterized Type I IFN which like IFN-α, is produced primarily by plasmacytoid dendritic cells in response to viral infection [11]. We therefore tested this IFN along with IFN-α to compare whether these substances produced similar inhibitory activity. Additionally, we compared the effects of the type II IFN, IFN-γ, which unlike type I

IFN, is produced primarily by T cells and NK cells and is known to bind receptors other than those for IFN-α and -β. As shown in figure 3, IFN-α once again significantly inhibited IL-13 secretion when using stimulating concentrations of IL-3 at 10 ng/ml. Inhibitory activity (~35%) was also observed with IFN-β, but only at the highest concentration tested (i.e. 100 U/ml), which did not reach significance ($p=0.08$). In contrast, there was no evidence for inhibition of IL-13 mediated by IFN-γ, which is consistent with the belief that suppression of this response is specific only for type I IFN.

Human basophils constitutively express IFN-α receptor mRNA

Both IFN-α and IFN-β are reported to signal through a common receptor, IFN-αR, for which the former IFN is thought to mediate greater activity. We therefore proceeded to determine whether basophils express this receptor. Figure 4 shows conventional RT-PCR results demonstrating that mRNA for IFN-αR is constitutively expressed in basophils. It is most important to note that these cells were not manipulated in any way other than being purified from blood. Furthermore, an amplicon of 765 bp was detected in all five of the basophil preparations and was qualitatively similar to that observed in a T cell preparation used as a positive control.

Effect of IFN-α on human basophil viability

There is evidence that therapeutic administration of IFN-α causes a sustained decrease in the circulating counts of various immune cells, including basophils [29]. We therefore thought it possible that a mechanism of IFN-α-mediated inhibition of basophil cytokine secretion might simply result from an ability to induce basophil cell death over a prolonged period of time. While this seemed unlikely in light of the anti-apoptotic and survival-enhancing properties that IL-3 has on basophils, we did test whether co-culture with IFN-α might reverse this effect of IL-3. As shown in figure 5, IL-3 markedly enhanced the survival of basophils cultured during a 5-day period, as expected, with approximately 50% of the cells viable at the end of 5 days compared to 15% survival in medium alone. More importantly, the addition of IFN-α (100 U/ml) did not significantly reduce this IL-3-mediated survival, especially during the first 72h of culture. Finally, there was some evidence that IFN-α alone provided slightly better survival conditions than those observed for cells cultured in medium alone, although no significant differences were identified. From these data it was concluded that IFN-α does not significantly affect basophil viability to the extent of inhibiting cytokine secretion.

Discussion

There have been many studies performed during the last 15 years showing that IL-3 is effective in priming human basophils for IgE-mediated responses, including both mediator release and cytokine secretion [23, 30-34]. More recently, IL-3 has also been shown to directly activate basophils for IL-13 secretion and to promote their survival in culture [6, 8, 35]. The enhancing effects of IL-3 are further substantiated by its ability to reverse the inhibitory effects that glucocorticosteroids have on mediator release [36]. In contrast, we show for the first time that IFN-α, a type I interferon, specifically inhibits the ability of IL-3 to promote cytokine secretion in basophils, an important finding considering it did not prevent cytokine produced in response to IgE-mediated stimulation alone (figure 1). In particular, we found that IFN-α reduced IL-3 priming of IgE-mediated IL-4 secretion and inhibited the capacity of IL-3 to directly induce IL-13 generation. Surprisingly, IFN-α did not affect the priming of IgE-mediated histamine release and LTC4 generation, suggesting that it targets an IL-3-dependent pathway(s) specific only for cytokine production. Furthermore, there was no evidence that IFN-α reversed the survival-enhancing capabilities of IL-3, which might otherwise account for its inhibitory effect on cytokine secretion (figure 5).

The biological significance of IFN-α inhibiting basophil cytokine secretion mediated by IL-3 is speculative at this time, yet we believe it relates to the Th1-like priming capabilities of this cytokine. For example, IFN-α has the ability to "prime" T cells for increased IFN-γ

production by making them more responsive to IL-12 through the up-regulation of STAT4 [37]. As mentioned, there are recent studies showing that plasmacytoid dendritic cells (PDC) account for the greatest production of this cytokine, particularly following viral infection or exposure to certain CpG-DNA oligonucleotides, both of which favor Th1-like responses. As a result, the data presented in this study are consistent with the Th1-promoting activities of IFN-α by showing that it suppresses the generation of IL-4 and IL-13, both of which are hallmark Th2-like cytokines. The fact that this occurs in basophils is most intriguing and suggests a more direct line of communication between basophils and PDC. To add to this complexity, both IL-4 and IL-13 are reported to inhibit IFN-α by PDC [38]. Therefore, basophils and PDC each secrete at least one regulatory cytokine that potentially down-regulates the cytokine-producing capabilities of the other. While it has long been suspected that such a control mechanism might exist between basophils and Th1 lymphocytes, we have yet to find a Th1-like cytokine possessing inhibitory activity equal to that mediated by IFN-α (JTS, unpublished data). In fact, this is exemplified in the present study by the observation that IFN-γ (a classic Th1 cytokine) had no inhibitory effect on the production of IL-13 by basophils treated with IL-3 (figure 3).

There is a long history of reports demonstrating that basophils of allergic subjects possess a primed phenotype and are hyper-responsive in nature [39-41]. More recent findings indicate that chronic allergen exposure plays a significant role in this increased responsiveness. For example,

we have shown that basophils spontaneously secrete IL-13 following repeated nasal allergen challenge [42]. While the simplest explanation for this finding is that allergen exposure induces cytokines (e.g. IL-3) that systemically prime basophils, it is intriguing to think that immune responses resulting in IFN-α production may, in fact, inhibit the priming of these cells. In this respect, the ability of IFN-α to inhibit priming is likely not limited to just the basophil. Indeed, several studies have presented evidence that IFN-α also decreases Th2 cytokine secretion (and mediator release) in eosinophils and mast cells [43-45].

While there is currently no *in vivo* evidence supporting IFN-α's role in preventing the priming of any cell type involved in allergic inflammation, there are clinical studies showing that its expression is reduced in allergic disease compared to non-allergic conditions. In a retrospective study involving 88 children, Bufe, et al. reported a significant correlation between the presence of an atopic phenotype and reduced levels of IFN-α. Approximately 2 to 3-fold lower levels of IFN-α were produced by mononuclear cells obtained from subjects diagnosed with allergic asthma compared to normal controls. Interestingly, this reduction was only evident in allergic asthmatics with measurable allergen-specific IgE, whereas normal levels of IFN-α were produced by PBMCs of children presenting with non-atopic asthma. At least one study has also reported reduced serum levels of IFN-α in a patient presenting with atopic dermatitis [46]. Accordingly, these clinical observations have led to the belief that IFN-α plays a significant role in reducing the atopic state. It therefore follows that its ability to

antagonize the enhancing effects of IL-3 on basophil IL-4 and IL-13 secretion accounts, in part, for this negative regulation.

Clinical case studies arising from IFN-α therapy also support the concept that this cytokine is an important modulator of Th1- and Th2- type immune responses. For example, there have been reports that injections of IFN-α have led to exacerbation of psoriasis and/or autoimmune conditions, both of which are characterized by Th1-like responses [47, 48]. As noted above, IFN-α has been used with success in treating neoplastic conditions such as hypereosinophilia, mastocytosis, and chronic myelogenous leukemia (CML). All of these involve the uncontrolled growth of immune cells for which growth factors (such as IL-5, SCF, and IL-3, respectively) likely have a positive influence. The high doses of IFN-α used in treating these conditions are thought to play a role in blocking the growth-enhancing capabilities of these growth factors [29]. It therefore seems possible that this is related to our observations of IFN-α inhibiting IL-3 priming for cytokine production. However, it is important to note that the inhibitory concentrations of IFN-α used in our study (i.e. 10-100 U/ml) are well below the estimated serum concentration (~750 U/ml) achieved with minimal therapeutic doses (e.g. $3x10^6$ units). Whether low-dose IFN-α therapy would be suitable for allergic disease remains to be seen, although at least one study has successfully used this cytokine in treating steroid-resistant asthma [49].

Finally, it is difficult at this time to predict the mechanism(s) by which IFN-α inhibits

IL-3-mediated cytokine secretion in basophils. Once again, the inhibition was specific for cytokine secretion and not mediator release. Therefore, the recent discoveries regarding the role of IL-3 in modulating mediator release by affecting various intracellular pathways may not fully apply to our findings [50, 51]. Messenger RNA for the IFN-αR subunit of the common type I IFN receptor was detected in basophils and at levels comparable to those found in T cells (figure 4). Thus, it does seem likely that the IFN-α used in this study mediated signaling through this receptor. This may further explain why some inhibitory activity was observed using IFN-β, which is also known to bind IFN-αR. It is currently not known whether any of the other variants of IFN-α, or other type I IFN's (e,g, IFN-ω) [9] possess similar repressive activity on basophil cytokine secretion.

In summary, these data show that IFN-α is capable of suppressing the enhancing effects IL-3 has on basophil production of IL-4 and IL-13, thus representing the first description of a cytokine possessing this activity. Significantly, this was only true for IL-4 and IL-13 production, as priming for histamine release and LTC4 synthesis was unaffected. These findings are consistent with the Th1-like promoting activities of IFN-α, suggesting that this cytokine plays a critical role in down-modulating allergic inflammation.

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Figure Legends

Figure 1. Effect of IFN-α on the IgE-mediated secretion of IL-4 (panel a), LTC4 (panel b), and histamine (panel c) with and without IL-3 priming. Basophils were cultured with and withoutIL-3 (100 pg/ml) and increasing concentrations of IFN-α. Cells were activated after 18h with the addition of anti-IgE antibody (10-20 ng/ml). Supernatants were harvested 4h later for concurrent measures of histamine, LTC4, and IL-4. Results are the average±s.e.m. of 4 experiments using cells from different donors. (*) denotes statistical significance ($p<0.05$).

Figure 2. Effect of IFN-α on the IL-3 –mediated production of IL-13. Basophils were cultured with the indicated concentrations of IL-3 and/or IFN-α. Supernatants were harvested after 18-20h incubation and analyzed for IL-13 protein by ELISA. The results from 6-8 experiments are expressed as the mean percentage ± s.e.m. of the control responses obtained in the absence of IFN-α, which were 20±9, 49±11, and 107±23 pg/10^6 basophils using IL-3 at 0.1, 1, and 10 ng/ml, respectively. (*) denotes statistical significance ($p<0.05$).

Figure 3. Inhibition of IL-3-mediated IL-13 secretion by type I vs. type II IFN. Basophils were stimulated with IL-3 (10 ng/ml) alone or in the presence of the indicated concentrations of IFN-α, IFN-β, and IFN-γ. Supernatants were harvested after 18-20 h incubation and analyzed

for IL-13 protein by ELISA. The results from 5 experiments are expressed as the mean percentage± s.e.m. of the control response with IL-3 alone, which was 102±32 pg/10^6 basophils. (*) denotes statistical significance.

Figure 4. Expression of type I IFN receptor (IFN-αR) in human basophils. Total RNA was extracted from basophil (exceeding 97% purity) and T cell pellets immediately after isolation from blood. Conventional RT-PCR was performed as described in the materials & methods. Shown is a gel image of the amplicons generated from 5 different basophil preparations and one T cell sample. HPRT expression is shown for comparison.

Figure 5. Effect of IFN-α on basophil survival *in vitro*. Basophils were cultured in medium alone or with the indicated concentrations of IFN-α (100 u/ml) and/or minimal IL-3 (100 pg/ml). Cell viability was assessed at the time points shown. Values represent the mean±s.e.m. of 3 experiments.

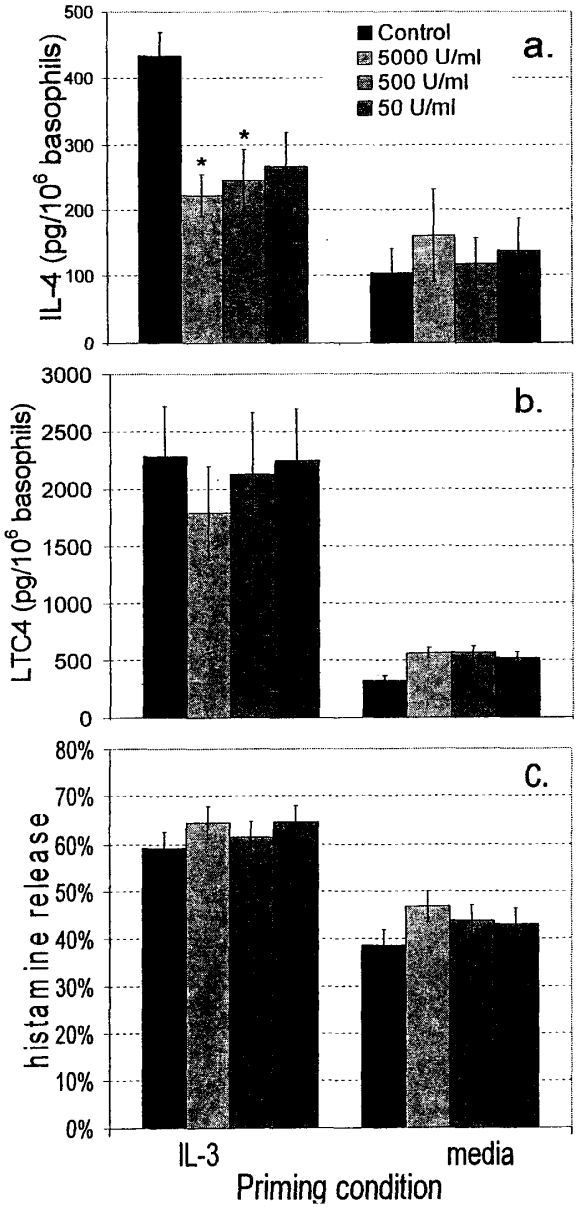

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Figure 1

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Figure 2

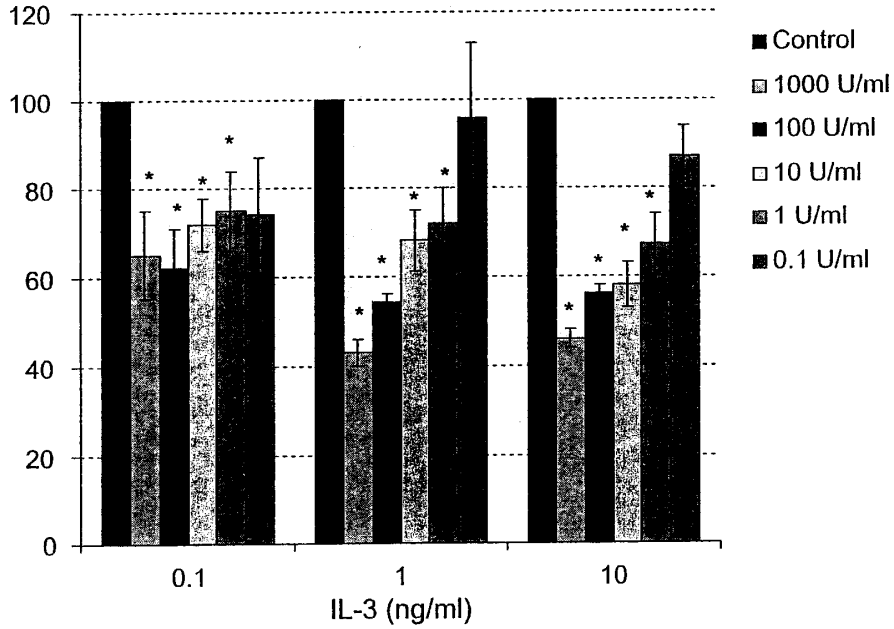

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Figure 3

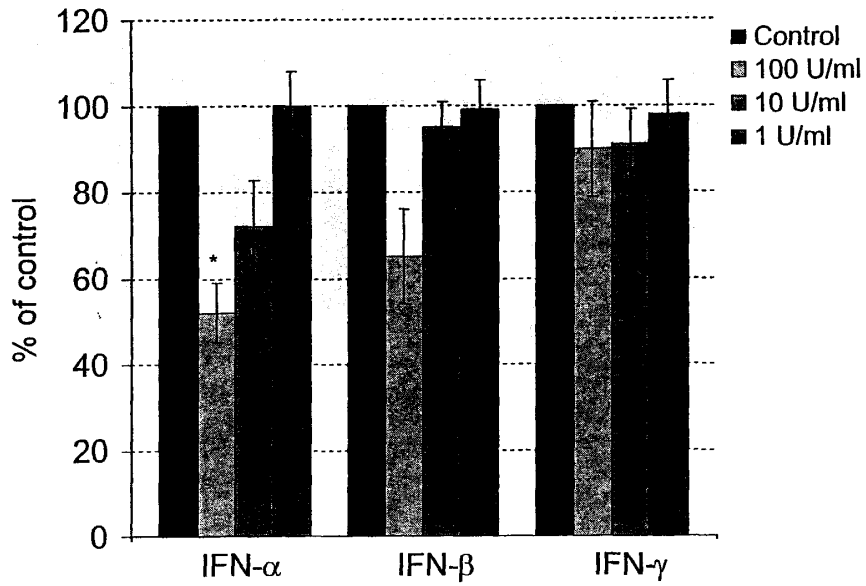

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Figure 4

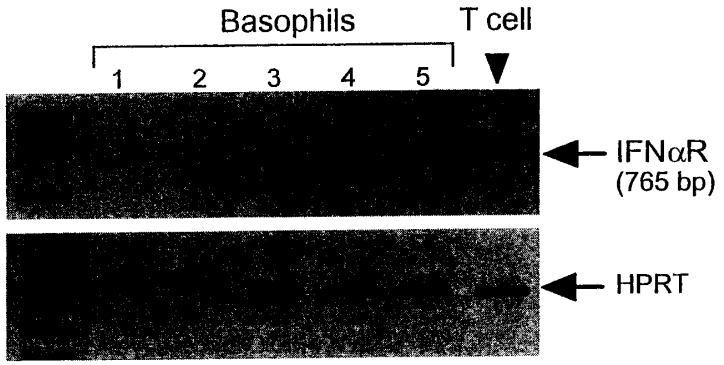

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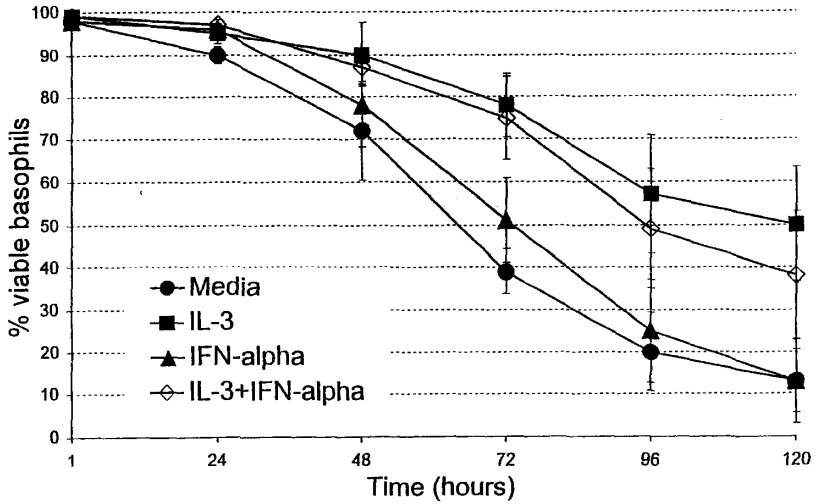
Figure 5



Expression of Toll-Like Receptor 9 in Human Basophils: A Potential cell Target for CpG-DNA

Running title: TLR9 expression and function in human basophils

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Summary

Unmethylated CpG-DNA and oligonucleotides containing CpG motifs (CpG-ODN) stimulate potent innate immune responses and have therapeutic potential in attenuating the Th2-mediated effects associated with allergic disease. Expression of Toll-like receptor 9 (TLR9), which is described exclusively in plasmacytoid dendritic cells (PDC) and B-cells, plays an important role in this immunomodulation as it is the putative receptor for CpG-DNA. We show here that TLR9 mRNA expression is found at high levels in human basophils, with relative levels comparable to those in PDC. Most significantly, basophils constitutively expressed more TLR9 protein, than did PDC, when assessed by both flow cytometry and Western blot. Pretreatment with IL-3 enhanced TLR9 expression in basophils and markedly up-regulated protein for this receptor in PDC to levels approaching those found on basophils. Evidence for functional TLR9 in basophils was demonstrated by the ability of CpG-ODN to activate NFκB (p65 subunit) for nuclear localization. Finally, basophils pretreated 16h with CpG-ODN secreted 25-50% less IL-4 and IL-13 when subsequently activated through the IgE receptor. These data suggest that while CpG-ODN/TLR9 interactions induce Th1 cytokine production in PDC they also help attenuate allergic responses by inhibiting Th2 cytokine generation from basophils.

Introduction

Unmethylated CpG motifs found in bacterial DNA stimulate strong innate immune responses both *in vitro* and *in vivo* and are characterized, in part, by the production of Th1 cytokines (e.g. IL-12, IFN-α) (1). These findings have provided the basis for using oligodeoxynucleotides containing specific CpG motifs (CpG-ODN) as immunostimulatory sequences (ISS) in allergen immunotherapy, offsetting the Th2 responses that are hallmark in allergic disease (2). While the exact mechanisms for this immunomodulation remain poorly defined, immune cells expressing Toll-like receptor 9 (TLR9) are likely to play a pivotal role, as this molecule is the putative receptor for CpG-DNA (3). To date, the presence of functional TLR9 in human cells is reportedly confined to plasmacytoid dendritic cells (PDC) and B-cells. Other cells, including T-lymphocytes, monocytes, dendritic cells of monocyte origin, and natural killer (NK) cells all seemingly lack TLR9 mRNA expression and fail to respond directly to CpG-ODN (4-6).

Human basophils have gained greater recognition in recent years for their capacity to generate large quantities of IL-4 and IL-13 in addition to releasing histamine and LTC4, all of which are hallmark in allergic inflammation (7). They share with PDC the unique distinction of expressing high levels of IL-3 receptor (CD123), through which IL-3 plays an important role in the development, maturation, and function of both cell types (7-9). This relationship prompted our investigation of whether basophils, like PDC, might express TLR9. As presented here, our findings show a striking amount of this receptor in basophils, with protein levels remarkably greater than those concurrently detected in PDC isolated from blood. Most importantly, CpG-ODN showed inhibitory activity against the IgE-mediated secretion of IL-4 and IL-13, indicating

that DNA oligos, while inducing Th1 cytokine production in PDC, may additionally help attenuate allergic responses by inhibiting Th2 cytokine generation from basophils.

Materials and Methods

Special Reagents

The following were purchased: crystallized human serum albumin (Calbiochem-Behring Corp, La Jolla, CA, USA); Piperazine-N,N'-bis-2-ethanesulfonic acid (PIPES), FBS, and crystallized BSA (Sigma Chemical Co, St Louis, MO, USA); gentamicin, Iscove's modified Dulbecco's medium (IMDM) , and nonessential amino acids,100x stock (Life Technologies, Inc, Grand Island, NY, USA); Percoll (Pharmacia Biotec, Inc, Piscataway, NJ); recombinant human IL-3 (Biosource, Inc. Camarillo, CA, USA); Rat monoclonal anti-TLR9 conjugated with phycoerythrin (PE) and its isotype/PE-conjugated control (e-Bioscience, San Diego, CA); anti-NFκB (p65 subunit) from Transduction Laboratories (San Diego, CA). Phosphorothioated ODN's were synthesized by Invitrogen (Grand Island, NY). Specific sequences described elsewhere included: PS2006, 5'- TCGTCGTTTTGTCGTTTTCGTGTT-3' and its GpC control, PS2083, 5'TGCTGCTTTTGTGCTTTTGCTGTT-3' (10).

Special Buffers and media

10x PIPES buffer (250 mM PIPES, 1.10 mM NaCl, 50 mM KCL, pH to 7.3 with 10 N NaOH) was stored at 4°C as a stock solution. PIPES/albumin/glucose (PAG) was made by diluting 1 part 10x PIPES with 9 parts deionized water; it containing 0.003% human serum albumin and 0.1% D-glucose. PAG-EDTA additionally contained 4 mM EDTA. Isotonic Percoll (referred to in this article as 100% Percoll) was made by mixing 1 part 10x PIPES with 9 parts Percoll. Solutions of 55% and 61% were made by mixing the appropriate amounts of 1x PIPES with 100% Percoll.

Conditioned medium (C-IMDM) consisted of IMDM supplemented with 5% heat-inactivated (56°C for 30 min) FBS, 1x nonessential amino acids, and 5 μg/ml gentamicin, pH 7.4.

Cell preparation and culture with CpG-ODN

Peripheral blood, anticoagulated with 10 mM EDTA, was taken with informed consent from healthy volunteers in accordance with protocols approved by the Western Institutional Review Board (WIRB). In some instances, residual cells of normal donors undergoing leukopheresis were used. Basophil-enriched cell (BEC) and basophil-depleted cell (BDC) suspensions were prepared on double Percoll gradients (1.075/1.081 g/ml), as previous described (11). Basophils within the BEC suspension were further purified to purities exceeding 96% (96 to >99%) using negative selection involving reagents from StemCell Technologies (Vancouver, Canada) combined with LS cell separation columns from Miltenyi Corporation (Auburn, CA), as described (12). Enumeration of basophils was done using Alcian-blue staining (13).

Basophils were cultured with CpG-ODN in C-IMDM containing low IL-3 (100 pg/ml) for 16 hours prior to activation for an additional 4h with anti-IgE antibody. Supernatants were measured for histamine release, IL-4 and IL-13 protein, as previously described (14).

PDC were enriched from BDC suspensions using the BDCA-4 positive selection kit, also from Miltenyi, with cell separation performed using LS columns attached to a Midi MACs magnet. Non-PDC were removed using 4 washes with buffer. PDC were then collected by removal of the column from the magnet and gently plunging it with buffer. Although not presented in this manuscript, cells isolated in this manner showed dendrite formation indicative of PDC following culture in IL-3 and produced high levels of IFN-α (>1ng/10^6 cells) during a 24h incubation upon activation with CpG-ODN.

Flow Cytometry

Direct staining for TLR9 protein was attained using an anti-TLR9 antibody conjugated with PE (e-Bioscience, San Diego, CA) and involved methods previously described for the detection of intracellular cytokines (15). Flow cytometry was performed using a FACSCalibur machine.

Qualitative and Quantitative RT-PCR

Total RNA was isolated from basophils and PDC ($0.5\text{-}1.0\text{x}10^6$ for each cell type) using the RNAzol protocol (Tel-test Inc., Friendswood, TX, USA). Methods for conventional and real-time reverse transcription (RT) and polymerase chain reaction (PCR) have been described elsewhere (16). Primers and/or probes were checked for specific sequences within the TLR9 gene (Accession No. AB045180). TLR9 primers for conventional RT-PCR were: forward primer sequence: 5'- TTATGGACTTCCTGCTGGAGGTGC-3' and reverse primer sequence: 5'-CTGCGTTTTGTCGAAGACCA-3', as reported (5). Hypoxanthine phosphoribosyl transferase (HPRT) was used for housekeeping gene expression as described elsewhere (17). Resolution of the cDNA products after amplification (35 cycles) was done in 3% agarose gels. Imaging was performed with an Electrophoresis Documentation and Analysis System 290 (Kodak Scientific Imaging Systems, New Haven, Conn.). One-step real-time (quantitative) RT-PCR was performed using an ABI PRISM 7700 thermocycler (Applied Biosystems, Foster City, CA). Primer/probe combinations for TLR9 have been published elsewhere (6) and included: forward primer 5'-GGACCTCTGGTACTGCTTCCA-3', reverse primer 5'-AAGCTCGTTGTACACCCAGTCT-3' and Probe 5'-CTGCAGGTGCTAGACCTGTCCCGC-3'. The reporter dye was 6-carboxy-fluorescein and the quencher was 6-carboxy-tetramethyl-rhodamine. Delta CT's were determined

by normalization to 18s rRNA using a commercially available kit (Applied Biosystems, Foster City, CA).

Western blot analysis

TLR9 protein expression was additionally determined by Western Blot analysis, as was the sub-cellular localization of NFκB following basophil activation with CpG-ODN. For TLR9 protein, both basophil and PDC whole cell lysates (using $5x10^5$ cells for each) were prepared by adding an equal volume of sample buffer (NOVEX, San Diego, CA) containing 5% 2-mercaptoethanol to cells resuspended in 1x PIPES buffer. For sub-cellular NFκB localization, basophils (at $3x10^6$/condition) were stimulated with CpG-ODN (or controls) for 1h in C-IMDM. Nuclear extracts were then prepared using a commercially available kit (Active Motif, Carlsbad, CA) and were diluted with an equal volume of sample buffer. Electrophoresis, protein transfer, and immunoblotting were performed as previously described (12) using the rat monoclonal anti-TLR9 and monoclonal NFκB (p65 subunit) antibodies noted above. Horseradish peroxidase-conjugated anti-rat and anti-mouse secondary antibodies were used for visualization with enhanced chemiluminescence.

Results

Expression of TLR9 mRNA in human basophils

Figure 1 shows RT-PCR results testing for TLR9 mRNA expression in basophil suspensions exceeding 99% purity. Conventional RT-PCR analysis indicated that basophils isolated from blood constitutively expressed mRNA for TLR9 (panel a). This somewhat surprising observation prompted further investigation using real-time RT-PCR to provide a more quantitative analysis comparing the relative levels of TLR9 mRNA in basophils with those detected in PDC. As seen in panel B, quantitative RT-PCR confirmed that the levels of TLR9 message expression in basophils from 10 subjects were comparable to those measured in 2 different PDC preparations after normalizing to 18s rRNA.

Constitutive expression of intracellular TLR9 protein in human basophils

Although few studies have actually demonstrated the presence of TLR9 protein in PDC or B cells, there is a body of evidence suggesting that this receptor is intracellular rather than expressed on the cell surface. Therefore, we next examined the expression of TLR9 protein using flow cytometric techniques for both intracellular and extracellular staining, and compared the levels of this receptor in basophils to that found in PDC. Since both cell types functionally respond to IL-3, we further investigated whether this cytokine plays a role in modulating the expression of TLR9 in these cells. Inserted in Figure 2 is a representative histogram showing that basophils stained for TLR9 using a PE-conjugated monoclonal antibody. Background staining with an isotype-matched control antibody is also shown for comparison. In basophils isolated from 7 subjects, the average mean fluorescence intensity (MFI) for intracellular TLR9 in

untreated cells (i.e. baseline) was 6 ± 0.5 (figure 2). Remarkably, this level of staining in basophils was over 6-fold greater than the baseline TLR9 protein barely detectable in PDC isolated from the same subjects. An18 h incubation in medium alone produced little change in TLR9 staining for either cell type. However, both cell types, when maintained in medium containing IL-3 (10 ng/ml), showed increased TLR9 protein expression. This was most significant for PDC in which the MFI increased to an average of 8±2, or to levels comparable to those seen in basophils (i.e 10±2). It is important to note that the MFI values for TLR9 in both cell types markedly decreased to levels barely above background when saponin was omitted from the staining solution, suggesting that this receptor is predominantly intracellular (data not shown).

Further analysis using immunoblotting confirmed the presence of TLR9 protein in basophils. Figure 3A shows prominent bands approximately 120 kd in size that were detected using anti-TLR9 antibody in whole cell lysates from four different basophil preparations. For comparison, panel B of this figure shows lysates prepared from 4 different PDC preparations using equivalent numbers of cells (5 x 10^5), clearly suggesting that qualitatively less TLR9 is found in these cells compared to that detected in basophils. However, in panel C, the 120 kd band scarcely visible in the lysates of PDC cultured 18 h in medium alone or in the presence of 0.1 ng/ml IL-3 is markedly increased in the same cells treated with 10 ng/ml IL-3. Overall, both the flow cytometry and immunoblotting results show that TLR9 is constitutively found in basophils and at relatively higher levels than those found in PDC. In addition, these results are the first to show that IL-3 plays an important role in regulating TLR9 protein levels in PDC and basophils.

CpG-ODN induces nuclear localization of NFκB in human basophils

A common downstream signaling event resulting from most TLR/ligand interactions is the activation and nuclear localization of NFκB (18). Therefore, we investigated the possibility of active TLR9 in basophils by testing whether CpG-ODN induces nuclear localization of NFκB. Figure 4 shows a representative experiment for which the nuclear extracts of cells treated 1h with the CpG oligo PS2006 showed immunoreactivity for the p65 subunit of NFκB, as assessed by Western blot analysis. The result was similar to that observed with equal numbers of basophils treated with PMA/Ionomycin (P/I) used as a positive control. In contrast, the p65 subunit was notably reduced in extracts of cells treated 1h in medium alone or with PS2083, a GpC control for the PS2006 oligo. All conditions showed immunoreactivity for a nonspecific band (NS) indicating equal loading of the nuclear extracts.

Effects of CpG-ODN on IgE-mediated histamine release and cytokine secretion

Since the NFκB experiments indicated functional responses using CpG-ODN, we next tested whether this oligo affected basophil secretion in response to IgE-mediated stimulation. Basophils were pretreated with several concentrations of PS2006 for 16h. Low levels of IL-3 (100 pg/ml) were added to these cultures in order to maintain basophil responsiveness to subsequent stimulation with anti-IgE. As show in figure 5, PS2006 equally inhibited the secretion of IL-4 and IL-13 by up to ~40% at concentrations between 8-200 nM. However, at higher concentrations, this oligo actually augmented histamine release, while having less inhibitory effect on cytokine. The effect on histamine release was, in part, due to the oligo alone inducing a small amount of histamine at these concentrations (panel B). In contrast, PS2006 did not induce cytokine release when used alone (data not shown).

Discussion

The results presented here show that human basophils express significant levels of TLR9, making this cell type a potential target of CpG-DNA activation. Until now, studies in humans have emphasized TLR9 expression in PDC and B-lymphocytes, since these cells express high levels of mRNA for this receptor and respond to CpG-ODN (4-6). However, using PDC as a positive control, we also detected TLR9 mRNA in basophils using quantitative RT-PCR analysis. To our surprise, we found relatively equal ratios of TLR9/18s rRNA in both cell types (Figure 1). Moreover, our results indicate that basophils may very well express more TLR9 protein than do PDC. Without additional treatment (other than their purification from blood) basophils constitutively expressed more protein for this receptor than did PDC, when analyzed using either flow cytometry or Western blot analysis (figures 2 and 3, respectively). In contrast, the expression of TLR9 protein was not apparent in PDC immediately isolated from blood (figure 2). While it remains possible that the positive selection protocol necessary for purifying PDC may account for this disparity, there is currently no evidence to suggest that engagement of the BDCA-4 antigen during purification causes a down-regulation in TLR9 protein expression.

We further believe that our results are the first to substantiate the importance of IL-3 in regulating TLR9 expression, particularly in PDC. As noted above, there are many studies showing that IL-3 plays a critical role in the development, maturation and function of both basophils and PDC. In fact, this relationship between the two cell types was, in part, the impetus for testing whether basophils might share with PDC the expression of TLR9. However, while IL-3 helped to maintain TLR9 protein expression in basophils, it had only a modest effect in up-regulating this receptor in these cells. In contrast, PDC required incubation with IL-3 in order to

achieve the levels of TLR9 protein found in basophils. Consequently, the constitutive expression of TLR9 in basophils supports the belief that these cells not only participate in innate immune responses, but may very well be a primary target for CpG-ODN mediated immunomodulation.

Another important observation is that TLR9, unlike any of the other TLR described thus far, is reported to be intracellular (18). Our study supports this concept, since staining for TLR9 protein was only evident following membrane permeabilization with saponin. This was true for both basophils and PDC. While it remains possible that IL-3, CpG-ODN or some other substance might promote cell surface expression of TLR9, this hypothesis was not investigated in the study presented here.

We can only speculate at this time as to the biological relevance of TLR9 expression in basophils, but there is indication that CpG-DNA affects the function of these cells. At relatively low concentrations (8-200 nM), the PS2006 oligo inhibited IgE-mediated secretion of IL-4 and IL-13 from basophils (figure 5). Since PS2006 at 100 nM also resulted in the nuclear localization of NFκB, it seems possible that activation of this transcription factor is partly responsible for this inhibition. In fact, this belief is supported by our previous work showing that phorbol myristate acetate (PMA), well known for its ability to activate NFκB in many cell types including basophils, inhibited IL-4 secretion from basophils stimulated with either anti-IgE or calcium ionophore (17). It is important to emphasize, however, that the inhibition of IL-4 and IL-13 by PS2006 was comparatively modest (25-50%) and occurred during a 16 h pre-incubation. This may indicate that the oligo indirectly inhibited the secretion of these cytokines in basophils by inducing some other factor(s) that act in an autocrine fashion. While this hypothesis requires additional investigation, we do feel that it is independent of IFN-α and IL-12, as we have yet to detect these cytokines in basophils following treatment with PS2006 (JTS, unpublished results).

It also remains possible that CpG-ODN other than PS2006 (a so-called type B or B cell-specific ODN) will differentially affect basophil function, much like what has been described for both PDC and B-cells (1, 19, 20). Nevertheless, our findings do suggest that CpG-ODN negatively regulate Th2 production in basophils allegedly though interactions involving TLR9. This observation, combined with our present understanding of what occurs in PDC and B-lymphocytes, may provide insight into the mechanisms behind the Th1-like promoting activities associated with CpG-DNA that may have therapeutic potential.

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Figure Legends

Figure 1. Qualitative and quantitative analysis of TLR9 mRNA Expression in human basophils. Total RNA was extracted from basophils and PDC ($5x10^5$-$1x10^6$) immediately isolated from blood. *A*, Gel image of conventional RT-PCR results for TLR9 using basophils from 5 different donors showing relative expression to the housekeeping gene, HPRT. *B*, Quantitative real-time RT-PCR results comparing TLR9 mRNA expression in basophils and PDC. Mean ± s.e.m. of 10 basophil preparations using different donors and the minimum and maximum values from 2 different PDC preparations.

Figure 2. Intracellular TLR9 protein in basophils and PDC using flow cytometry. Basophils and PDC isolated from blood were either untreated (i.e. baseline) or were cultured 18h in IL-3 (10 ng/ml) or medium alone. Cells were fixed with 4% paraformaldehyde and prepared for intracellular TLR9 staining using single color flow cytometry as described in *Materials and Methods*. The inserted histogram shows staining with anti-TLR9 (and its isotype control) in a representative preparation of untreated (i.e. baseline) basophils. Bar graph results are presented as the means ± s.e.m of 7 basophil and 6 PDC preparations from different donors.

Figure 3. Western blot analysis for TLR9 protein in basophils and PDC. Whole cell lysates from 4 different donors were prepared using $5x\ 0^5$ untreated basophils (panel A) and an equal number of untreated PDC (panel B). Panel C is of a separate experiment using cells from a single donor for which whole cell lysates were prepared from untreated PDC and PDC cultured 18 h in IL-3 (0.1 and 10 ng/ml) or medium alone ($5x10^5$ cells for each condition). Electrophoresis and

immunoblotting were performed as described in *Materials and Methods*. The single band detected at 120kd denotes TLR9 protein.

Figure 4. Nuclear localization of NFκB in basophils following CpG-ODN activation. Basophils ($3x10^6$/condition) were cultured 1h in either medium alone, 100 nM PS2006 (CpG), 100 nM PS2083 (GpC), or PMA and ionomycin (10 and 500 ng/ml, respectively, P/I). Nuclear extraction was then performed followed by electrophoresis and Western blotting for the p65 subunit of NFκB, as described in *Materials and Methods*. NS denotes non-specific protein and suggests equal loading of nuclear extracts. Shown is a representative of 3 experiments.

Figure 5. Effect of pretreatment with CpG-ODN on IgE-mediated histamine release and cytokine secretion. Basophils isolated from blood were incubated 16h in the presence of IL-3 (100 pg/ml) and the indicated concentrations of PS2006. Cells were then activated with anti-IgE (10-20 ng/ml) and incubated 4h for mediator release and cytokine content. *A*, Values are the mean ± s.e.m. (n=3). Control release of IL-4 and IL-13 with anti-IgE (no PS2006) was 382±46 and 173±39 pg/10^6 basophils, respectively. *B*, Control release of histamine when PS2006 and anti-IgE were used separately.

Figure 1
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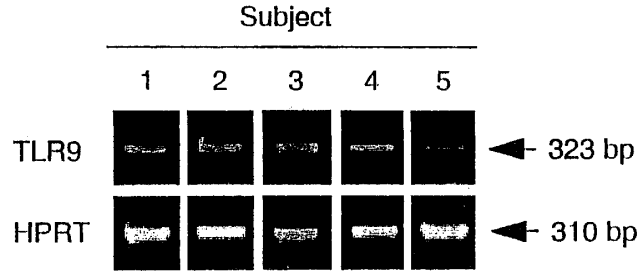
A.



B.

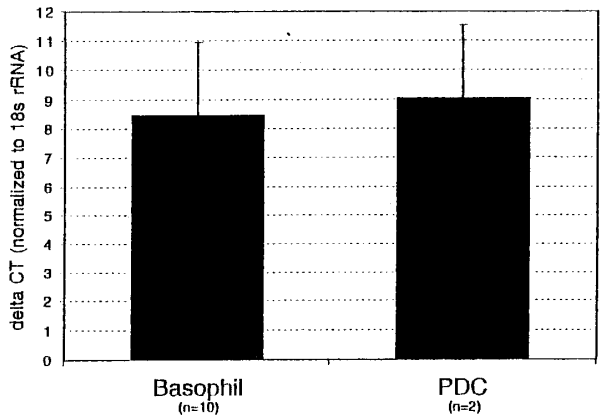


Figure 2
Chen, et al.

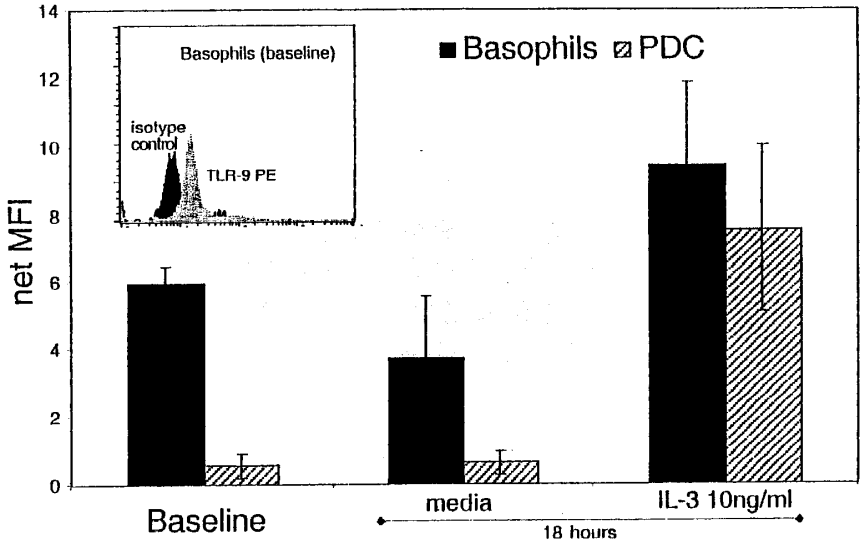


Figure 3
Chen, et al.

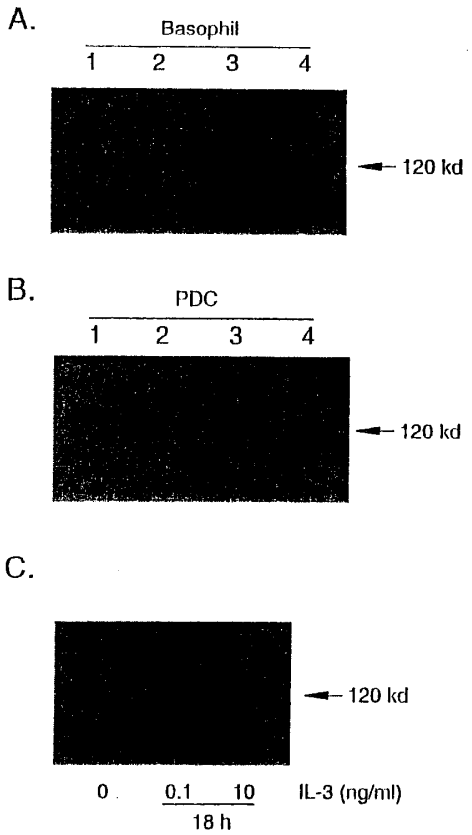
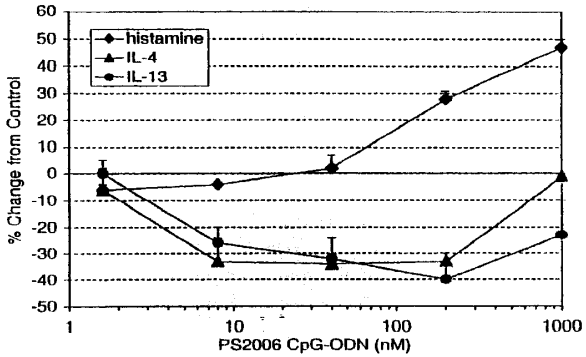
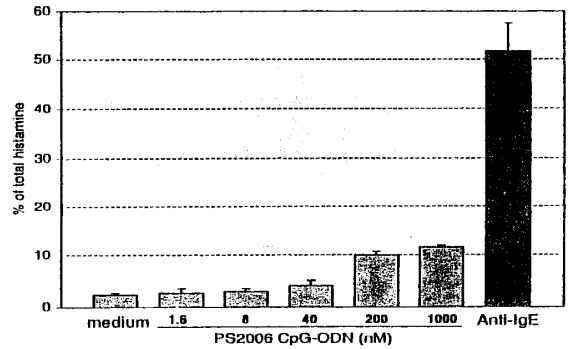


Figure 5
Chen, et al.

A



B



Title: New Approaches of Immunotherapy: Allergen Vaccination with Immunostimulatory DNA

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Abstract

Allergen immunotherapy, first introduced in the early part of the twentieth century, is widely practiced despite several limitations. Considerable effort has been devoted to developing new anti-allergic therapeutic vaccines that, compared with conventional allergen immunotherapy, improve efficacy, decrease the time required to achieve effect, reduce inconvenience and enhance safety. Increased understanding of the molecular biology of allergic respiratory inflammation has led to the development of therapeutic vaccines that potentially suppress or arrest the disease process in asthma or allergic rhinitis. This paper addresses a specific DNA vaccine approach in which highly active immunostimulatory DNA are conjugated to the principal allergenic moiety of a relevant aeroallergen (i.e., ragweed Amb a 1). ISS-DNA based vaccines have proven to drive Th1-type immune responses and to prevent Th2- type allergic inflammations in various models. A study group at Johns Hopkins University has recently completed the first human study of AIC, a new therapeutic vaccine composed of a conjugate of Amb a 1 and 1018 ISS, in patients with allergic rhinitis. The results of this study demonstrated that AIC was 185 fold less allergenic than licensed ragweed extract used for conventional immunotherapy when evaluated by quantitative intradermal skin titration methodology. Furthermore, AIC was 50-fold less likely to trigger histamine release from human basophils. Mixture of ISS and Amb a 1 did not

alter the histamine release compared with Amb a 1 alone. Placebo-controlled, randomized, double-blinded clinical trial recently conducted at the Johns Hopkins revealed encouraging clinical efficacy and safety of AIC in ragweed allergic rhinitic subjects. AIC may offer the potential for an improved safety and efficacy for immunotherapy. Additional trials to further evaluate long-term safety, immunologic effect, and therapeutic efficacy of AIC for ragweed-induced allergic rhinitis and asthma are ongoing.

Introduction

Conventional immunotherapy is a well-recognized method that aims to modulate an allergic patient's immune response through administration of increasing doses of an extract, comprised of the aeroallergens to which the patient has been demonstrated to be allergic, and thereby to attenuate or eliminate the patient's symptoms. Allergen immunotherapy was first introduced in the early part of the 20th century and has been widely practiced. Controlled clinical trials have demonstrated the therapeutic efficacy and detailed the favorable immunologic changes associated with allergen immunotherapy for the treatment of allergic rhinitis, asthma, and venom sensitivity.[1-7]

However, this approach is saddled with a number of encumbrances including the need for frequent dosing over years, which impacts upon patient compliance; the need to administer a relatively large dose of the immunizing agent to achieve control of symptoms; and the potential for clinically significant allergic reactions to the treatment. Therefore, considerable effort has been devoted to developing improved therapeutic vaccines for treatment of allergic diseases to a) improve efficacy; b) decrease the time required to achieve effect; c) reduce the inconvenience and hence improve compliance with immunization regimens; and d) enhance safety.

Allergen dose is limited by systemic reactions to the respective allergens. Thus, efforts have largely been directed at decreasing the "allergenicity" (i.e. their potential

for inducing an allergic reaction) of the antigens while maintaining or heightening their immunogenicity (i.e. their ability to induce a beneficial immunologic response). Although various chemical modifications of allergens have been attempted, the end-result has been that allergenicity and immunogenicity have either decreased or increased in tandem.

Certainly, an allergenic vaccine with reduced allergenicity, but maintained immunogenicity, which could be given in a few doses, would have important therapeutic implications. Millions of patients with poorly controlled allergic rhinitis and asthma would be candidates for such a form of immunomodulation.

This paper addresses a specific DNA vaccine approach in which highly active immunostimulatory phosphorothioate oligodeoxyribonucleotide moieties (ISS-ODN) are conjugated to the principal allergenic moiety of a relevant aeroallergen (e.g., ragweed Amb a 1).[8 9] This adjuvant approach may prove to be highly effective at directing the immune response toward up-regulation of a more favorable Th1 phenotypic expression to counter-balance the untoward Th2-driven pro-inflammatory allergic process.

Allergy is a Th2 disease

To improve materials for immunotherapy, it is important to understand the pathophysiology of the disease process. Upon allergen exposure in a susceptible individual, there is an acute allergic response reflective of IgE-dependent cell activation resulting in release of histamine, leukotrienes, and other mediators from mast cells and basophils.

In addition, allergen is processed by antigen-presenting cells that display allergen in association with class II human leukocyte antigen molecules. If appropriate co stimulatory signals are also induced, the result is T-lymphocyte activation with induction of Th2 cells that produce "pro-inflammatory" cytokines (e.g., IL-4, IL-5, and IL-13). The identification of transcription factors controlling Th1 and Th2 development further support the Th2 hypothesis because GATA3 is over expressed and T-bet is under expressed in the asthmatic airway. These cytokines induce recruitment of inflammatory cells such as eosinophils and basophils with the resultant development of airway inflammation.[10-13]

Mechanism of Conventional Immunotherapy

The allergy group at Johns Hopkins took an early lead in examining the mechanisms by which allergen immunotherapy effects clinical improvement. As increasing doses of extract are injected, there is an initial elevation in the levels of

both IgG- and IgE-specific antibodies. With continuing therapy, IgG levels further increase and plateau whereas antigen-specific IgE titers gradually decline toward pretreatment levels and are not boosted by subsequent environmental exposure to the allergen.[14-17]Induction of IgG antibodies is a predictor of clinical success albeit clinical benefit is not likely to be achieved until doses are large enough to risk anaphylaxis.

Nasal allergen challenge techniques have been used to demonstrate that immunotherapy shifts Th2 cell responses toward Th1 activation. Immunized patients demonstrate less immediate mediator release in their nasal secretions and less late-phase eosinophil migration.[18-21] Nasal biopsies of grass-immunized rhinitic patients after grass pollen extract challenge show a significant increase in mRNA for specific Th1 cytokines [IFN-γ and IL-12]. This is paralleled by a significant reduction in allergen-induced accumulation of total numbers of CD4+ T cells and eosinophils in nasal mucosa. Interestingly, these techniques showed little down-regulation of the expression of cytokines from Th2 cells (e.g., IL-4, IL-5).[22-25] Tangential to these findings, Secrist et al cultured peripheral blood mononuclear cells from allergic patients receiving maintenance grass immunotherapy and demonstrated a significant decrease in allergen-induced IL-4 synthesis when these cells were exposed to allergen in vitro.[26]

These studies demonstrate that immunotherapy has the potential to down-regulate not only the immediate-phase allergic reaction, but also late-phase T-cell-mediated responses. The problem lies in administering a dose large enough to induce the desired changes without causing intolerable allergic side effects. Towards this goal, Dynavax Technologies Corporation has developed a novel product consisting of ragweed allergen (Amb a 1) linked to immunostimulatory phosphorothioate oligodeoxyribonucleotide. The Amb a 1 immunostimulatory oligonucleotide conjugate (AIC) induces an enhanced, ragweed-specific Th1-type response in mice in comparison to either Amb a 1 alone or Amb a 1 + alum.[8,27]

Background for adjuvant approaches

Several molecules including lipopolysaccharides, aluminum hydroxide salts, and Freund's adjuvant have long been observed to possess immunostimulatory properties with enhanced response to antigen. Attempts have been made to capitalize on this observation to improve a vaccine's immunogenicity through an enhanced adjuvant effect. Tokunaga and his colleagues made the initial discovery of the adjuvant effect of bacterial DNA with their study of the active components of Freund's adjuvant. [28-30] Krieg et al subsequently identify unmethylated CG dinucleotides, so-called CpG motifs, to be responsible for the immunostimulatory effect of bacterial DNA.[31] Bacterial DNAs are now known to possess immunostimulatory properties that are

absent in vertebrate DNA. These properties are related to the higher frequency of CpG motifs and to the absence of cytosine methylation in bacterial as opposed to vertebrate DNA, which otherwise would abolish the immunostimulatory activity. [29,32,33] The effects of bacterial DNAs can be mimicked using synthetic oligonucleotides (ODN), thus allowing a more accurate definition of the bacterial DNA immunostimulatory sequences. Early research studies initially identified optimal ISS sequences containing palindromic hexamers based on the general formula of:

5'-purine-purine-CG-pyrimidine-pyrimidine-3' (e.g., 5'-GACGTC-3', 5'-AGCGCT-3' and 5'-AACGTT-3'). [31] More recent work has extended to the identification of different types of CpG ODN based on their activities to plasmacytoid dendritic cells (PDC), NK cells and B cells. CpG-A ODN contains phosphodiester backbones and is particularly effective at inducing production of interferon-α from plasmacytoid dendritic cells and activating NK cells whereas CpG-B ODN possesses phosphorothioate backbones that enhance B cell proliferation and immunoglobulin production but little PDC and NK cell stimulation.[34,35]

ISS-ODN INDUCE TH1-TYPE AND INHIBIT TH2-TYPE IMMUNE RESPONSES

Not until recently that Toll-like receptor-9 (TLR-9) is identified to be the receptor of CpG.[36] CpG binds TLR-9 and signaling through MyD88 and activate NF-κB

similar to other Toll-like receptors.[37] TLR-9 was found to express predominantly on PDC and B cells in human though some cell types yet to be tested.[38,39] CpG-A ODN strongly stimulates PDC to produce high amount of IFN-α and IL-12 thus activate IFN-γ producing NK cells and γδ T cells, which drive immune cells toward Th1 responses[35,40-43]

Low concentration of CpG-ODN leads to great increase in B cell proliferation, antigen-specific immunoglobulin secretion and IL-6 production [31,44] Exposure of tonsil B cells to CpG-ODN induces a concentration- and time-dependent up-regulation of the activation markers CD23, CD25, CD40, CD54, CD80, CD86 and HLA-DR, and counteracted IgE production induced by IL-4. [45] CpG directly induces T-bet expression and inhibits IgG1 and IgE switching in B cells induced by IL-4 and CD40 signaling.[46]

Pre-administration of CpG ODN prevents allergen-induced Th2 responses, including IL-5 production, eosinophilic airway inflammation, airway hyper-reactivity as well as chronic airway remodeling in murine models [47-51]

Therefore, CpG, or ISS-ODN, appears to stimulate the innate immune system to produce cytokines that drives adaptive immune system toward Th1-type and inhibit Th2-type immune responses, which suggests a potential clinical utility for CpG in the immunotherapy of allergic diseases.

Effects of ISS-ODN based allergen vaccine

Conjugation of allergen with ISS-ODN (AIC) was developed because it was believed that AIC may be more immunogenic and less allergenic than simple cocktail of allergen mixed with ISS-ODN.[8] The immunomodulatory effect of direct linkage or conjugation of allergen with ISS is demonstrated in Figure 1. This graph shows that in a murine animal model immunization with a conjugate of Amb a 1 and 1018 ISS (AIC) promotes IgG2a antibody production that is significantly greater than that observed with either allergen mixed with ISS, allergen + alum, or allergen alone. In contrast, an inhibition of IgE production has been a corollary finding to this "protective" IgG antibody response. These observations bear particular relevance when it is recognized that in the murine model IgG2a is a marker of induction of a T H1response; whereas IgE production is induced through TH2 mechanisms. Furthermore, these reciprocal IgG and IgE antibody responses are similarly reproducible in previously sensitized and not simply naive animals.[8,27] This proffers the opportunity to not only use this approach as prophylactic intervention but also to employ this therapeutic construct in sensitized individuals that are in the midst of an allergic diathesis.

AIC results in the preferential induction of naïve CD4+ T cells with differentiation toward a Th1 phenotypic profile. The corollary to this observation of a

down-regulation in Th2-mediated responses to allergen (e.g., IL-4, IL-5) is thought to be the result of macrophage or monocytic activation (IL-12, IL-18, IFN-α/β with constituent effects on Th1 activation (increased IFN-γ). [8,9,27] Figure 2 demonstrates in a murine model the specific up regulation of IFN-γ (Th1 profile) as a result of AIC immunization in contradistinction to the Th2 cytokine dysregulation that would otherwise be observed with allergen alone, allergen + alum, or allergen mixed with ISS but not linked to this specific adjuvant. Marshall et al[9] have made similar observations by means of in vitro studies of peripheral blood mononuclear cells obtained from ragweed-allergic human subjects. Indeed this work demonstrates that cells exposed to AIC produced significant IFN-γ (indicative of the Th1-response) with diminished production of IL-4/IL-5 (TH2-like profile).

Furthermore, Horner et al showed in a recent study that an optimal conjunction ratio of AIC lead to approximately 100 fold less allergenic than native allergen using mast cell degranulation method and marked reduction in anaphylactogenicity and Arthus reaction in murine models.[52]

Clinical applications with DNA vaccine

Our study group at Johns Hopkins has recently completed the first human safety study of the use of immunostimulatory DNA in ragweed-allergic rhinitic patients. This was a US FDA-defined clinical safety study that employed quantitative

intradermal endpoint skin test titration to assess the relative potency of the AIC product to its comparator (standardized ragweed extract). The results of this study demonstrated that AIC were 185 fold less reactive than licensed ragweed extract when evaluated by this quantitative intradermal skin titration methodology. Furthermore, AIC was 50-fold less likely to trigger histamine release from basophils. Mixture of ISS and Amb a 1 did not alter the histamine release compared with Amb a 1 alone.[53] Our first clinical trial with subcutaneous administration of AIC has demonstrated the ability of the DNA vaccine to induce ragweed- pecific IgG antibody production in ragweed-allergic patients. This is an important clinical observation since favorable clinical outcomes correlate with, and can be predicated by, induction of an IgG antibody titer.[54] Our observations that AIC exhibited fewer local reactions on skin testing, in comparison to licensed ragweed, suggests that this novel product may offer the potential for an improved safety profile for immunotherapy. Pilot placebo-controlled, randomized, double-blinded clinical study recently conducted at the Johns Hopkins revealed encouraging clinical efficacy and safety of AIC in ragweed allergic rhinitic subjects.[55] However, further trials on evaluating the optimal dosing, long-term safety, immunologic effect, and therapeutic efficacy of AIC as a treatment modality for ragweed-induced allergic rhinitis and asthma are needed.

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IMPORTANT: Please complete this form and bring to your appointment.

NAME: DATE OF BIRTH: APPT. DATE:

ALLERGY PROFILE

Please check the problems that you have or had: [ ] rhinitis or "hay fever" [ ] sinusitis [ ] asthma
[ ] food allergy [ ] drug allergy [ ] stinging insect allergy [ ] latex allergy [ ] eczema [ ] hives [ ] swelling

Please check the things that trigger or worsen any of the above: [ ] dust [ ] pollens [ ] molds
[ ] tobacco smoke [ ] fumes or perfumes [ ] dry or cold air [ ] exercise [ ] aspirin, ibuprofen, naproxen
[ ] animals, specify: [ ] food, specify: [ ] others, specify:

Have you experienced an **allergic or other adverse reaction to a medication**? [ ] No. [ ] Yes.

If yes, please list the date(s) of occurrence, the drug(s), and the side effect(s). Use extra sheet if needed.

Have you been **previously tested** for any form of allergy? [ ] No. [ ] Yes, skin test. [ ] Yes, blood test.
If yes, when was it done and which tests were positive?

Have you been **previously treated** for any of the above conditions? [ ] No. [ ] Yes, with allergy injections.
[ ] Yes, with medications. Please specify:

PAST MEDICAL AND SURGICAL HISTORY

Please check the medical conditions that you have or had and write the date when they were diagnosed.

[ ] Cataracts or Glaucoma	[ ] **Other medical conditions.** Specify and date:
[ ] Depression	
[ ] Diabetes	
[ ] Heart disease	
[ ] Hepatitis or any liver disease	[ ] **Surgical operations.** Specify and date:
[ ] High blood pressure	
[ ] Peptic ulcer or acid reflux	
[ ] Thyroid disease	

CURRENT MEDICATIONS

Please write the name and dosage of ALL prescription and over-the-counter drugs that you are taking.

1	5
2	6
3	7
4	8

FAMILY MEDICAL HISTORY

Please write the medical conditions that members of your family have or had.

[ ] living [ ] deceased Father:

[ ] living [ ] deceased Mother:

Siblings/others:

Number of brothers & sisters: ___ Number of children: ___ Please list any family member who has or had
[ ] rhinitis or "hay fever": [ ] asthma: [ ] allergic dermatitis:

ENVIRONMENTAL HISTORY

Please list the cities/states where you have resided in from birth to present (including dates):

How old is your present HOME? _____ How long have you lived there? _____
Is your home a [ ] single family house? [ ] rowhouse? [ ] townhouse? [ ] apartment? [ ] mobile home?
Does your home have [ ] central or forced warm air heating? [ ] radiator heating? [ ] central air-conditioning?
[ ] window air-conditioning units? [ ] humidifier? [ ] any damp area? [ ] cockroaches? [ ] any smoker?

Does your **bedroom** have [ ] wall-to-wall carpeting? [ ] hardwood flooring? [ ] area rugs? [ ] stuffed toys?
Do you use [ ] foam pillows? [ ] fiber-filled pillows? [ ] feather pillows? [ ] bed mattress? [ ] box spring?
[ ] water bed? [ ] dust mite-proof pillow covers? [ ] dust mite-proof bed covers?

Do you have fur-bearing pets? [ ] No. [ ] Yes. If yes, please specify how many and what kind? _____
Since when? _____ Do the pets go into your bedroom? [ ] No. [ ] Yes, sometimes. [ ] Yes, often.

Please list your **hobbies**:

WORK HISTORY

Occupation: Dates and places and of employment:

Does your workplace have [ ] central air-conditioning? [ ] window air-conditioning units?
Are you exposed to chemicals, irritants, latex products, or animals at school or at work? [ ] No. [ ] Yes.
If yes, please specify:

PERSONAL AND SOCIAL HISTORY

Do you or did you smoke cigarettes? [ ] No. [ ] Yes, until now. [ ] Yes, but stopped in _____.
If yes, how many packs do you or did you smoke per day and for how many years?

Do you drink alcoholic beverages? [ ] No. [ ] Yes. If yes, how much and how often?

REVIEW OF SYSTEMS

Please check all that apply to you.

Constitutional: [ ] prolonged fever or chills [ ] significant weight loss or weight gain [ ] chronic fatigue
Eyes: [ ] itchy and watery eyes [ ] use of eye glasses or contacts **ENT:** [ ] hearing loss [ ] ear ache
[ ] runny nose [ ] stuffy nose [ ] frequent sneezing [ ] postnasal drip [ ] frequent nose bleeding
[ ] mouth ulcers [ ] hoarseness [ ] tongue or throat swelling [ ] difficulty in swallowing
Resp: [ ] breathlessness [ ] wheezing [ ] chest tightness [ ] cough [ ] bloody sputum [ ] abnormal X-ray
CV: [ ] chest pain [ ] palpitations [ ] heart murmur [ ] abnormal heart rhythm [ ] abnormal EKG
GI: [ ] abdominal pain [ ] heartburn [ ] jaundice [ ] diarrhea [ ] constipation [ ] bloody stools
GU: [ ] pain in urination [ ] frequent urination [ ] difficulty in urination [ ] incontinence [ ] kidney stones
Heme: [ ] anemia [ ] easy bruising [ ] abnormal bleeding **Skin:** [ ] frequent hives [ ] rash [ ] itching
Musculoskeletal: [ ] joint pain [ ] joint swelling [ ] joint stiffness [ ] muscle pain
Neuro: [ ] seizures [ ] weakness [ ] numbness **Psych:** [ ] feeling depressed [ ] difficulty in sleeping

If everything listed above is negative, please check here: [ ]

Additional details or comments:

Physician's initial/signature: _____

The Johns Hopkins Center for Asthma and Allergic Diseases

Skin Test Diagnostic Profile

Inhalants I

Name: _____

History No. _____

Physician: _____

Date _____ Technician _____

Check	Extract	ID	Puncture Test Conc Size	Intradermal #1 (1:100) Size	A	B	Intradermal #2 (1:10) Size	A	B
	Time ON READ								
	TREES								
☐	Tree Mix I:	T1	W / E						
☐	Oak *Quercus*	T2	W / E						
☐	Beech *Fagus*	T3	W / E						
☐	Maple *Acer*	T4	W / E						
☐	Elm *Ulmus*	T5	W / E						
☐	Tree Mix II:	T6	W / E						
☐	Birch *Betula*	T7	W / E						
☐	Ash *Fraxinus*	T8	W / E						
☐	Sycamore *Platanus*	T9	W / E						
☐	Poplar *Populus*	T10	W / E						
☐	Tree Mix III:	T11	W / E						
☐	Hickory *Carya*	T12	W / E						
☐	Walnut *Juglans*	T13	W / E						
☐	Mulberry *Morus*	T14	W / E						
☐	Juniper *Juniperus*	T15	W / E						
	3° Trees:								
☐	Heaven *Ailanthus*	T16	W / E						
☐	Privet *Ligustrum*	T17	W / E						
☐	Willow *Salix*	T18	W / E						
☐	Box Elder *Acer*	T19	W / E						
☐	Alder *Alnus*	T20	W / E						
☐	Diluent P-50% gly ID-Alb.-saline	C1	W / E						
☐	Histamine P-10 mg/ml ID-0.1 mg/ml	C2	W / E	3+		3+			
☐	Glycerine ID-6%	C3	W / E						

Check	Extract	ID	Puncture Test Conc Size	Intradermal #1 (1:100) Size	A	B	Intradermal #2 (1:10) Size	A	B
	Time ON READ								
	GRASSES								
☐	Timothy *Phleum*	G1	W / E						
☐	Orchard *Dactylis*	G2	W / E						
☐	Bermuda *Cynodon*	G3	W / E						
☐	June *Poa*	G4	W / E						
☐	Knotgrass *Paspalum*	G5	W / E						
☐	Johnson *Sorghum*	G6	W / E						
☐	Vernal *Anthox*	G7	W / E						
☐	Brome *Bromus*	G8	W / E						
☐	Velvet *Holcus*	G9	W / E						
☐			W / E						
	WEEDS								
☐	Ragweed, short *Ambrosia*	W1	W / E						
☐	Plantain *Plantago*	W2	W / E						
☐	Weed Mix I:	W3	W / E						
☐	Pigweed *Amaranthus*	W4	W / E						
☐	Lambsquarters *Chenopodium*	W5	W / E						
☐	Sage, Mugwort *Artemisia*	W6	W / E						
☐	Sheep Sorrel *Rumex*	W7	W / E						
☐			W / E						
☐			W / E						
☐			W / E						

Notes: ☐ = Routine Screen

Criteria A (Puncture and Intradermal)

0 no discernible wheal
1+ <1/2 histamine diameter
2+ ≥1/2 histamine: < histamine diameter
3+ =size of histamine control ± 1 mm
4+ >histamine diameter: <2x diameter
5+ ≥2x histamine control

Criteria B (Intradermal only)

	Erythema (mm)	Wheal (mm)
0	<5	<4
+-	5-10	5-10
1+	11-20	5-10
2+	21-30	5-10
3+	31-40	10-15
4+	41-50	>15

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The Johns Hopkins Center for Asthma and Allergic Diseases

Skin Test Diagnostic Profile

Inhalants II

Name: _____

History No. _____

Physician: _____

Date _____ Technician _____

Check	Extract	ID	Puncture Test Conc	Intradermal #1 (1:100)		Intradermal #2 (1:10)		
	Time ON READ							
			Size	Size	A	Size	A	B
	MOLDS							
☐	Mold Mix I:	M1						
☐	Aspergillus	M2						
☐	Penicillium	M3						
☐	Mucor	M4						
☐	Fusarium	M5						
☐	Mold Mix II:	M6						
☐	Alternaria	M7						
☐	Helminthosporium	M8						
☐	Cladosporium (Hormodendrum)	M9						
☐	Botrytis	M10						
	2° Molds:							
☐	Agaracus	M11						
☐	Ustilago	M12						
☐	Fuligo	M13						
☐	Calvatia	M14						
☐	Aurobasidium	M15						
☐	Epicoccum	M16						
	3° Molds:							
☐	Candida	M17						
☐	Trichophyton	M18						
☐								
☐	Diluent P-50% gly ID-Alb.-saline	C1						
☐	Histamine P-10 mg/ml ID-0.1 mg/ml	C2		3+		3+		
☐	Glycerine ID-9%	C3						

Check	Extract	ID	Puncture Test Conc	Intradermal #1 (1:100)		Intradermal #2 (1:10)		
	Time ON READ							
			Size	Size	A	Size	A	B
	HOUSEHOLD INHALANTS							
☐	Dust Mite/D.farinae	H1						
☐	Dust Mite/D.Pter.	H2						
☐								
☐	Cockroach	H3						
☐								
	EPIDERMALS							
☐	Cat	E1						
☐	Dog	E2						
☐	Horse	E3						
☐	Guinea Pig	E4						
☐	Rat	E5						
☐	Mouse	E6						
☐	Rabbit	E7						
☐	Gerbil	E8						
☐	Hamster	E9						
☐	Cattle	E10						
☐								
☐								
☐								
☐								
☐								

Notes: ☐ = Routine Screen

Criteria A (Puncture and Intradermal)

0 no discernible wheal
1+ <1/2 histamine diameter
2+ ≥1/2 histamine: < histamine diameter
3+ =size of histamine control ± 1 mm
4+ >histamine diameter: <2x diameter
5+ ≥2x histamine control

Criteria B (Intradermal only)

	Erythema (mm)	Wheal (mm)
0	<5	<4
+-	5-10	5-10
1+	11-20	5-10
2+	21-30	5-10
3+	31-40	10-15
4+	41-50	>15

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The Johns Hopkins Center for Asthma and Allergic Diseases

Skin Test Diagnostic Profile

Drugs & Chemicals

Name: _____

History No. _____

Physician: _____

Date _____ Technician _____

Check	Reagent	ID	Intradermal Test 1	Intradermal Test 2	Intradermal Test 3	Intradermal Test 4	Intradermal Test 5	Provocation Test	Comments
	Time ON READ								
			Size A B	Size A B	Size A B	Size A B	Size A B		
	Penicillin*		Puncture Test (1X)	1X	10X	50X			
☐	Penicilloyl Polylysine (PPL)	D1	W / E						
☐	Minor Determinant Mixture (MDM)	D2	W / E						
	Local Anesthetics		Puncture Test (1:1000)	1:1000	1:100	1:10	Concentrate		
☐	Xylocaine with methylparaben	D3	W / E						
☐	Procaine (Novocain)	D4	W / E						
☐	Mepivacaine (carbocaine)	D5	W / E						
☐	Xylocaine without preservative	D6	W / E						
	Insulins		0.001 u/ml	0.01 u/ml	0.1 u/ml	1.0 u/ml	10 u/ml		
☐	Ultrapure Pork	D7	W / E						
☐	Humulin	D8	W / E						
☐	Beef/Pork	D9	W / E						
	General Anesthetics		1:10000	1:1000	1:100	1:10	Concentrate		
☐	Pancuronium Bromide (Pavulon) 2 mg/ml	D10	W / E						
☐	Curare (Tubocurarine) 3 mg/ml	D11	W / E						
☐	Succinylcholine (Quelicin Suxamethonium) 20 mg/ml	D12	W / E						
☐	Thiopental 25 mg/ml	D13	W / E						
	Other Drugs*		Puncture Test (1:1000)	1:1000	1:100	1:10	Concentrate		
☐	Bactrim (IV Prep)	D14	W / E						
☐			W / E						
☐			W / E						
☐			W / E						
☐			W / E						
☐			W / E						
☐	Diluent P-50% gly ID-Alb.-saline	C1	W / E						
☐	Histamine P-10 mg/ml ID-0.1 mg/ml	C2	W 3+ / E	3+					
☐	Codeine P-15 mg/ml ID- 2 mg/ml	C3	W / E						

* To be performed in duplicate. Record average of duplicates.

Criteria A (Puncture and intradermal)	Criteria B	(Intradermal only) Erythema (mm)	Wheal (mm)
0 no discernible wheal>diluent	0	<5	<4
1+ <1/2 histamine diameter	+/-	5-10	5-10
2+ ≥1/2 histamine: < histamine diameter	1+	11-20	5-10
3+ =size of histamine control ± 1 mm	2+	21-30	5-10
4+ >histamine diameter: <2x diameter	3+	31-40	10-15
5+ ≥2x histamine control	4+	41-50	>15

Form 104
Revised 8/10/92

The Johns Hopkins Center for Asthma and Allergic Diseases

Skin Test Diagnostic Profile

Insects/Stinging & Biting

Name: _____

History No. _____

Physician: _____

Date _____ Technician _____

Check	Reagent	ID	Puncture Test Size	Puncture Test A	Puncture Test B	Intradermal Test 1 Size	Intradermal Test 1 A	Intradermal Test 1 B	Intradermal Test 2 Size	Intradermal Test 2 A	Intradermal Test 2 B	Intradermal Test 3 Size	Intradermal Test 3 A	Intradermal Test 3 B	Intradermal Test 4 Size	Intradermal Test 4 A	Intradermal Test 4 B
	Time ON																
	READ																
	Hymenoptera±		0.1 µg/ml			0.01 µg/ml			0.1 µg/ml			1.0 µg/ml			3.0 µg/ml		
☐	Honey Bee	I1	W														
			E														
☐	Yellow Jacket	I2	W														
			E														
☐	White-faced Hornet	I3	W														
			E														
☐	Yellow Hornet	I4	W														
			E														
☐	Polistes Wasp	I5	W														
			E														
☐			W														
			E														
	3˚ Other Insects*		1:100			1:1,000			1:100			1:10			Concentrate		
☐	Fire Ant	I6	W														
			E														
☐	Blackfly	I7	W														
			E														
☐	Deerfly	I8	W														
			E														
☐	Mosquito	I9	W														
			E														
☐			W														
			E														
☐			W														
			E														
☐	Diluent P-50% gly ID-Alb.-saline	C1	W														
			E														
☐	Histamine P-10 mg/ml ID-0.1 mg/ml	C2	W		3+			3+									
			E														
☐	Codeine P-15% mg/ml ID-2 mg/ml	C3	W														
			E														

Notes: ☒ = Routine Screen
Standard dilutions are unshaded.

± Venom Proteins

* Whole body extracts

Criteria A (Puncture and Intradermal)

0	no discernible wheal>diluent
1+	<½ histamine diameter
2+	≥½ histamine: <histamine diameter
3+	= size of histamine control ± 1mm
4+	>histamine diameter: <2x diameter
5+	≥2x histamine control

Criteria B (Intradermal only)

	Erythema (mm)	Wheal (mm)
0	<5	<4
+/-	5-10	5-10
1+	11-20	5-10
2+	21-30	5-10
3+	31-40	10-15
4+	41-50	>15

Form 103
Revised 11/8/85

The Johns Hopkins Center for Asthma and Allergic Diseases

Skin Test Diagnostic Profile

Foods

Name: _____

History No. _____

Att. Physician: _____

Date _____ Technician _____

Time	ON		Time	ON		Secondary Food List			
	READ			READ					
Check	Extract	ID	PunctureTest	Check	Extract	ID	PunctureTest		
			Size A				Size A	ID	Common Name	ID	Common Name
☒	**Primary Panel** 1:10 w/v			☐	**Other Foods** 1:10 w/v			F30	Apple	F80	Onion
☐	Egg	F1	W / E	☐				F31	Apricot	F81	Orange
☐	Peanut	F2	W / E	☐				F32	Banana	F82	Pea, black-eyed
☐	Milk	F3	W / E	☐				F33	Barley	F83	Peach
☐	Soy	F4	W / E	☐				F34	Bean, Lima	F84	Pear
☐	Fish Mix: Halibut, Cod, Mack	F5	W / E	☐				F35	Bean, Navy	F85	Pepper, Green
☐	Shell Fish Mix: Clam, Crab	F6	W / E	☐				F36	Blackberry	F86	Pepper, Black
☐	Chicken	F7	W / E	☐				F37	Blueberry	F87	Pineapple
☐	Pork	F8	W / E	☐				F38	Broccoli	F88	Potato, Sweet
☐	Beef	F9	W / E	☐				F39	Buckwheat	F89	
☐	Potato, white	F10	W / E	☐				F40	Cabbage	F90	Raspberry, Red
☐	Tomato	F11	W / E	☐				F41	Cantaloupe	F91	Sesame seed
☐	Strawberry	F12	W / E	☐				F42	Carrot	F92	Shellfish, Clam
☐	Corn	F13	W / E	☐				F43	Casein	F93	Shellfish, Crab
☐	Pea	F14	W / E	☐				F44	Cauliflower	F94	Shellfish, Oyster
☐	Greenbean	F15	W / E	☐				F45	Celery	F95	Shellfish, Scallop
☐	Rice	F16	W / E	☐				F46		F96	Shellfish, Shrimp
☐	Rye	F17	W / E	☐				F47	Cherry	F97	Spinach
☐	Cocao bean Chocolate	F18	W / E	Provocation Tests:				F48	Cinnamon	F98	
☐	English Walnut	F19	W / E					F49	Coffee	F99	Squash
☐	Wheat	F20	W / E	Notes:				F50		F100	Turkey, Meat
☐	Oat	F21	W / E					F51	Cranberry	F101	Turnip
☒	Diluent P-50% gly ID-Alb.-saline	C1	W / E					F52	Cucumber	F102	Vanilla
☒	Histamine P-10 mg/ml ID-0.1 mg/ml	C2	W / E 3+					F53	Fish, Cod	F103	Watermelon
☒	Codeine P-15 mg/ml ID- 2 mg/ml	C3	W / E					F54	Fish, Flounder	F104	
								F55		F105	
								F56	Fish, Mackerel	F106	Fish, Black Bass
								F57	Fish, Tuna	F107	Fish, Catfish
								F58	Garlic	F108	Lobster, Maine
								F59	Ginger	F109	Fish, Perch
								F60	Goat Milk	F110	Fish, Salmon
								F61	Grape, White	F111	Fish, Sardine/Herring
								F62	Grapefruit	F112	Fish, Lake Trout
								F63	Hops, Food		
								F64			
								F65	Lamb		
								F66	Lemon		
								F67	Lettuce		
								F68	Malt		
								F69	Mushroom		
								F70	Mustard, Food		
								F71	Nut, Almond - Food		
								F72	Walnut, Black - Food		
								F73	Nut, Brazil		
								F74	Nut, Cashew		
								F75	Nut, Coconut		
								F76	Nut, Filbert/Hazel		
								F77	Nut, Pecan		
								F78	Nutmeg		
								F79	Olive, green		

Criteria A (Puncture and Intradermal)

0 no discernible wheal
1+ <1/2 histamine diameter
2+ ≥1/2 histamine: < histamine diameter
3+ =size of histamine control ± 1 mm
4+ >histamine diameter: <2x diameter
5+ ≥2x histamine control

1 of 6

Solution vials provided
A B C D E (circle)
Date prepared ________
Comp: tree grass weed
mold animal mite

The Johns Hopkins Center for Asthma and Allergic Diseases
IMMUNOTHERAPY SCHEDULE

Patient's Name ____________________

Physician ____________________

Date ____________________

Instructions:

1. Frequency of injection:__________until maintenance dose #______achieved, then repeat dose #______every______weeks.
2. Patient should remain for observation for 30 minutes after each injection.
3. Dosage adjustments:
   A) Modify dosage accordingly for missed injections:
      1) Repeat last dose, if shot is 1 wk. overdue.
      2) Cut back by 1 dose #, if shot is 2 wks. overdue.
      3) Cut back by 2 dose #'s, if shot is 3 wks. overdue.
      4) Call "Center" if shot is 4 or more wks. overdue.
   B) Modify dosage for local swelling 12-24 hrs. post injection:
      1) Repeat same dose #, for wheal/swelling of >30mm (50¢ size) diameter.
      2) Cut back 1 dose # for wheal/swelling of >60mm (2 1/2") diameter.
   C) Modify dosage schedule accordingly for systemic reactions to injections:
      1) Cut back by 2 dose #'s for systemic reactions in vials A-D.
      2) Cut back by 3 dose #'s for systemic reactions in vial E.
   D) Call "Center" at 550-2313 if you are concerned about any reaction that occurred or dosage adjustment.

Recommended Schedule

Check	Dose No.**	Vial	Vol (ml)
☐	1	A (1:10,000)	0.1
☐	2	↓	0.2
☐	3	↓	0.4
☐	4	↓	0.8
☐	5	B (1:1,000)	0.1
☐	6	↓	0.2
☐	7	↓	0.4
☐	8	↓	0.8
☐	9	C (1:100)	0.1
☐	10	↓	0.2
☐	11	↓	0.4
☐	12	↓	0.8
☐	13	D (1:10)	0.1
☐	14	↓	0.2
☐	15	↓	0.4
☐	16	↓	0.8
☐	17	E (concentrate)	0.1
☐	18	↓	0.15
☐	19	↓	0.2
☐	20	↓	0.3
☐	21	↓	0.4
☐	22	↓	0.5
☐	23	↓	0.6
☐	24	↓	0.7
☐	25	↓	0.8
☐	26	↓	0.9
☐	27	↓	1.0

Record Injection Treatments Here

Date	**Dose #	Given By	Time	Arm		Systemic* Reaction		Local Induration >30mm at 12-24 hrs.		Pred. ____ Best ____ Cutoff ____ PRE	POST
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		
				R	L	Y	N	Y	N		

—PEFR—

	Date	Dose #	mm wheal/erythema
Initial dose Skin test			/

Treatment Facility/Physician

☐ JHAAC
☐ JHH-A
☐ JHH-P
☐ OTHER

*Systemic Reaction Notes
(append additional sheets for systemic reactions if required)

Date	Description	Onset (minutes)	Treatment	By

CCU 29 12/9/92

OVER FOR SOLUTION REORDER FORM

** Please record dose # (not volume of dose) for each injection given.

SOLUTION REORDER FORM

Name: _____
Date: _____

Location Seen
- ☐ JHAAC at FSK Medical Center
- ☐ JHH Adult Asthma & Allergy Clinic
- ☐ JHH Pediatric Asthma & Allergy Clinic

1. Solution refill request should be completed and returned at least **three weeks** in advance of need.

2. Mail the completed form to:

   Allergen Laboratory
   Johns Hopkins Asthma & Allergy Center
   Unit Office 6
   5501 Hopkins Bayview Circle
   Baltimore, Maryland 21224

3. The solutions should be mailed to:

   _____
   _____
   _____
   _____

4. Center policy requires that patients on allergy injection treatments be seen by a staff allergist at least yearly. Last or next appointment: _____. If necessary, call (410) 550-2300 for appointment.

Payment for renewal solutions must accompany this form. A check for $100 should be made payable to the Johns Hopkins Allergen Lab, or indicate charge card information below. BC/BS of Maryland (Major Medical), HMO, Medicare and Medical Assistance patients will have charges billed directly for them.

☐ Check attached

☐ BC/BS of MD
Major Medical

☐ HMO Billing
Referral Required (please attach)

☐ Medicare
I.D.# _____
Enroll. date _____

☐ Medical Assistance
M.A.# _____
Exp. date _____

☐ Charge card as follows:

- ☐ VISA
- ☐ Master Charge
- ☐ American Express

Card number _____ Expiration date _____

Signature _____

CCU 24 050290

附件十一

The Program Tracks of Johns Hopkins Asthma and Allergy Center

Program Description

The Division offers post-doctoral training designed to prepare well-qualified trainees with either an M.D. or Ph.D. for academic careers in allergy and immunology and, more generally, in inflammation research. The program is strongly oriented toward research training ranging from purely laboratory or bench investigation to clinical investigations or therapeutic studies. Three tracks are offered:

- **Allergy-Clinical Immunology**
  This program is ordinarily a three year commitment for physicians who have completed training in internal medicine and who desire sub-specialty allergy and immunology clinical training leading to board certification. An intensive supervised research program is included. The Division accepts both trainees who wish to prepare for a career in laboratory research and those who wish to prepare for investigation of patients in the clinical arena. During the first two years, approximately 50% effort is devoted to clinical training and conferences, with the remainder of time assigned to research instruction, closely supervised by one or more faculty members. During the second year, a mutual agreement is reached concerning the advisability of a third year of intensive research.
- **Research-Intensive Experience**
  For those physicians who have completed or are in the midst of clinical training in another specialty (e.g., pulmonary medicine, rheumatology, otolaryngology) and who do not seek formal clinical training in allergy and immunology, this program provides an intensive research experience. While these physicians may be assigned to one clinic session (1/2 day) per week throughout their tenure, the great majority of their time will be focused on laboratory or clinical investigation under the guidance of one or more faculty members. Designed for physicians committed to careers in biomedical research and wishing to pursue this goal with minimal clinical responsibilities,

this track is not intended for those desiring board certification in allergy and immunology.

- **Post-Doctoral Laboratory Research**

  This program provides laboratory research training experience for scientists with Ph.D.s, M.D.s or comparable advanced degrees. Such post-doctoral trainees will often apply to, and be accepted by the individual member of the Faculty with whom he or she desires to work. Applications for undesignated post-doctoral positions are also accepted. Such trainees will not ordinarily have clinical, teaching, or administrative responsibilities, except as may be mutually agreed upon prior to the Fellowship.

The Program tracks of Johns Hopkins Asthma and Allergy Center

Brief Description	Training Period(years)	No.of Positions Available/yr	Pre-requisites	Research Time	Years	Clinical Sessions(per week)	Consult Service (mo/yr)
Allergy Clinical Immunology	3	2	M.D.#	75% Lab or clinical research	1 2 3	4 2 1	3 2 *
Research intensive	2-3	varied	M.D.+	90% Lab or clinical research	1 2 (3)	1 1 1	* * *
Post-doctoral Lab research	2-3	varied	PhD, M.D. or equivalent	100% lab research	1 2 3	* * *	* * *

Residency training in internal medicine or pediatrics

+ Advanced clinical training in allied specialty

JULY 2003

ORIENTATION CONFERENCES

MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
	1	2 1:00 Prevalent and relevant outdoor allergens **Peter Creticos, M.D.** 2:00 Pathophysiology of rhinitis **Alvin Sanico, M.D.** 3:00 Pathopathology of asthma **Alkis Togias, M.D.**	3	4 **No Conferences**
7	8	9 1:00 Prevalent and relevant indoor allergens **Peyton Eggleston, M.D.** 2:00 Anaphylaxis and other allergic emergencies **Romi Saini, M.D.** 3:00 Consults: Do's & Don'ts **Bruce Bochner, M.D., N.F. Adkinson, M.D. & Staff**	10	11 1:00 Historical perspective on immunotherapy **Philip Norman, MD** 2:00 Clinical trials of immunotherapy in allergic rhinitis/asthma **Peter Creticos, M.D.**
14	15	16 1:00 Therapeutic decisions in asthma management **Peter Creticos, M.D.** 2:00 Acute and chronic sinusitis **Jean Kim, M.D., Ph.D.** 3:00 Methods to study allergic airways inflammation **Mark Liu, M.D.**	17	18 **No Conferences**
21	22	23 1:00 Drug hypersensitivity **N.F. Adkinson, M.D.** 2:00 Biology of IgG and IgE receptors **Romi Saini, M.D.** 3:00 Mast cell and basophil secretion **John Schroeder, Ph.D.**	24	25 1:00 Choosing a research project **N.F. Adkinson, M.D.** 2:00 Atopic dermatitis **Lisa Beck, M.D.** 3:00 **OPEN**
28	29	30 1:00 Genetic epidemiology of allergic diseases **Kathleen Barnes, Ph.D.** 2:00 HIV/radiation/hepatitis precautions and lab practices **Allen Myers, Ph.D.** 3:00 Optimizing aerosol delivery and deposition in human airways **Beth Laube, Ph.D**	31	

To Be Scheduled:

A) Annual fall weed walk

B) Practicums to be arranged independently with Nurse Manager for:

1) Nebulizer/metered dose inhaler/peak flow meter devices
2) NSAID's/sulfite challenge
3) Bronchoprovocation techniques

AUGUST 2003
ORIENTATION CONFERENCES

MONDAY	TUES	WEDNESDAY	THURS	FRIDAY
				1 1:00 Food and food additive hypersensitivity **Robert Wood, M.D.** 2:00 Transcriptional regulation of cytokine genes I **Vincenzo Casolaro, M.D. and Steve Georas, M.D.** 3:00 IgE biology and synthesis **Susan MacDonald, M.D.**
4	5	6 1:00 Th1 and Th2 paradigms **David Essayan, M.D.** 2:00 Airway smooth muscle physiology **Allen Myers, Ph.D.** 3:00 Chemokines and chemokine receptors **Cristiana Stellato, M.D., Ph.D.**	7	8 1:00 Cell adhesion molecules **Bruce Bochner, M.D.** 2:00 Diagnosis and treatment of urticaria and angioedema **Romi Saini, M.D.** 3:00 Interpretation of pulmonary function tests **Robert Wise, M.D.**
11	12	13 1:00 Animal models of allergic diseases **Brendan Canning, Ph.D.** 2:00 Signal transduction pathways-I **Donald MacGlashan, M.D., Ph.D. and Becky Vonakis, Ph.D.** 3:00 Signal transduction pathways-II **Donald MacGlashan, M.D., Ph.D. and Becky Vonakis, Ph.D.**	14	15 1:00 The use of mass spectrometry for detection and quantitation of mediators and markers of airway inflammation **Walter Hubbard, Ph.D.** 2:00 Anti-inflammatory actions of steroids **Robert Schleimer, Ph.D.** 3:00 Virus-immune regulation **Farhad Imani, Ph.D.**
18	19	20 NO CONFERENCES ABIM	21	22 1:00 Choosing a research project **N. Franklin Adkinson, M.D.** 2:00 Laboratory evaluation of the allergic patient **Robert Hamilton, Ph.D.** 3:00 Pharmacology of airways hyperreactivity **Brad Undem, Ph.D**
25	26	27 1:00 Research skills practicum: bench to bedside **Bruce Bochner, M.D., Peter Creticos, M.D. and Brendan Canning, Ph.D.** 2:00 Acute and chronic sinusitis **Jean Kim, M.D., Ph.D.**	28	29 1:00 OPEN 2:00 Insect venom sensitivity and treatment **David Golden, M.D.** 3:00 OPEN

To Be Scheduled:

A) Annual fall weed walk

B) Practicums to be arranged independently with Shirley Dixon and Sharon Pickett for:

1) Nebulizer/metered dose inhaler/peak flow meter devices
2) NSAID's/sulfite challenge
3) Bronchoprovocation techniques

JUNE 2003

JOHNS HOPKINS ASTHMA AND ALLERGY CENTER
5501 HOPKINS BAYVIEW CIRCLE, BALTIMORE, MARYLAND 21224
DIVISION OF CLINICAL IMMUNOLOGY

MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
2	3	4 FACULTY/FELLOW PHOTOGRAPHS 11:45 **Lunch** 12:00 am CTP 2B.65 "Sinus Polyposis" **Tao Lee, M.D.** 1:00 **Res. Conf. 2B.65** "Transgenic Modeling of IL-13 in the Lung" **Paul Elias, M.D.** 3:00 **FELLOWS FAREWELL RECEPTION**	5	6 8:00 a.m. **Hurd Hall** Medical Grand Rounds 12:30 **No Luncheon** 1:00 **ACI Conf 2B.65** "Allergy/Clinical Immunology Journal Club" **Bradley Undem, PhD**
9	10	11 11:45 **Lunch** 12:00 am CTP 2B.65 "Clinical Case Study" **Mark Scarupa, M.D.** 1:00 **Res. Conf. 2B.65** "IgE-dependent and IgE-independent Pathways of Human Mast Cell Activation" **Guha Krishnaswamy, M.D.** 2:00 **General Faculty Meeting** 4:00 **Clinical Faculty Meeting**	12	13 8:00 a.m. **Hurd Hall** Medical Grand Rounds 12:30 **Luncheon** 1:00 **ACI Conf 2B.65** "Urticaria (Autoimmune)" **Allen Kaplan, M.D.**
16	17	18 11:45 **Lunch** 12:00 am CTP 2B.65 "Rev: ABPA" **Tamara Perry, M.D.** 1:00 **Res. Conf. 2B.65** NO CONFERENCE	19	20 8:00 a.m. **Hurd Hall** Medical Grand Rounds 12:30 **Luncheon** 1:00 **ACI Conf 2B.65** "Aspirin Desensitization" **Paul Greenberger, MD**
23	24	25 NO CONFERENCE	26	27 8:00 a.m. **Hurd Hall** Medical Grand Rounds 12:30 **Luncheon** 1:00 **ACI Conf 2B.65** "Aspirin Respiratory Disease: Pathogenesis and Treatment" **Don Stevenson, M.D.**
30				

Key: **IC**-Immunology Council Mtg., **Mountcastle Auditorium, PCTB**; **CTP**-Clinical Teaching Program, **GR**-Grand Rounds, Hurd Hall, JHH; **ACI**-Allergy Clin. Immun.; **GFM**-General Fac. Mtg.; **CFM**-Clinical Fac Mtg., **RC**-Research Conference, **CAIR**- Comprehensive Allergy & Immun. Review. **5/30/03**

MAY 2003

JOHNS HOPKINS ASTHMA AND ALLERGY CENTER
5501 HOPKINS BAYVIEW CIRCLE, BALTIMORE, MARYLAND 21224
DIVISION OF CLINICAL IMMUNOLOGY

MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
			1	2 **8:00 a.m. Hurd Hall** Medical Grand Rounds **12:30 No Luncheon** **1:00 ACI Conf 2B.65** NO CONFERENCE
5	6 4:15 p.m. IC SPH – Rm. W2030 NO SEMINAR	7 11:45 Lunch 12:00 am CTP 2B.65 "Clinical Case Study" Tao Le, M.D. 1:00 Res. Conf. 2B.65 "Regulation of Chemokine Gene Expression by Hyaluronic Acid" Dr. Maureen Horton 2:00 CAIR Conference, 2B.74 3:00 Clinical Faculty Meeting	8	9 **8:00 a.m. Hurd Hall** Medical Grand Rounds **12:30 No Luncheon** **1:00 ACI Conf 2B.65** NO CONFERENCE 1:00 General Faculty Meeting
12	13 4:15 p.m. IC SPH – Rm. W2030 Dr. John O'Shea	14 11:45 Lunch 12:00 am CTP 2B.65 "Food Allergen Epitopes" Mark Scarupa, M.D. 1:00 Res. Conf. 2B.65 "Regulation of Pulmonary Inflammation" Dr. Dale Umetsu 2:00 CAIR Conference, 2B.74 3:00 General Faculty Meeting	15	16 **8:00 a.m. Hurd Hall** Medical Grand Rounds **12:30 No Luncheon** **1:00 ACI Conf 2B.65** "Allergy/Clinical Immunology Journal Club" Brendan Canning, PhD 2:00 INSERVICE EXAM
19	20 4:15 p.m. IC SPH – Rm. W2030 Dr. Martin Kast	21 NO CONFERENCES ATS MEETING	22	23 **8:00 a.m. Hurd Hall** Medical Grand Rounds **12:30 No Luncheon** **1:00 ACI Conf 2B.65** "Management of Non-Allergic Rhinitis" Michael Kaliner, M.D.
26	27 4:15 p.m. IC SPH – Rm. W2030 Dr. Wink Baldwin	28 11:45 Lunch 12:00 am CTP 2B.65 "Levalbuterol for Bronchodilation" Sally Joo, M.D. 1:00 Res. Conf. 2B.65 "Regulation of Eosinophil Survival by Siglec-8: Research Update" Dr. Esra Nutku 2:00 CAIR Conference, 2B.74	29	30 **8:00 a.m. Hurd Hall** Medical Grand Rounds **12:30 No Luncheon** **1:00 ACI Conf 2B.65** "Welch Updates" Holly Harden

Key: IC-Immunology Council Mtg., **Mountcastle Auditorium, PCTB**; CTP-Clinical Teaching Program, GR-Grand Rounds, Hurd Hall, JHH; ACI-Allergy Clin. Immun.; GFM-General Fac. Mtg.; CFM-Clinical Fac. Mtg., RC-Research Conference, CAIR- Comprehensive Allergy & Immun. Review.

This calendar is sent electronically each month. If you would like to be added to the list of recipients, please send your email address to: dtjones@jhmi.edu