I. Basic Immunology

A. Immune Mechanisms

1. Innate versus adaptive immunity

LANDMARK PUBLICATION:
Krieg AM, Yi A, Matson S et al
CpG motifs in bacterial DNA trigger B-cell activation.
Nature 1995;374:546-549.

Unmethylated CpG dinucleotides are more frequent in the genomes of bacteria and viruses than of vertebrates. We report here that bacterial DNA and synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides induce murine B cells to proliferate and secrete immunoglobulin in vitro and in vivo. This activation is enhanced by simultaneous signals delivered through the antigen receptor. Optimal B-cell activation requires a DNA motif in which an unmethylated CpG dinucleotide is flanked by two 5' purines and two 3' pyrimidines. Oligodeoxynucleotides containing this CpG motif induce more than 95 percent of all spleen B cells to enter the cell cycle. These data suggest a possible evolutionary link between immune defence based on the recognition of microbial DNA and the phenomenon of `CpG suppression' in vertebrates. The potent immune activation by CpG oligonucleotides has implications for the design and interpretation of studies using `antisense' oligonucleotides and points to possible new applications as adjuvants.

a. Natural Antimicrobial Agents

i. Releasable granule proteins

LANDMARK PUBLICATION:
Ong P
Endogenous Antimicrobial Peptides and Skin Infections in Atopic Dermatitis
N Engl J Med 2002; 347:1151-1160

Background The innate immune system of human skin contains antimicrobial peptides known as cathelicidins (LL-37) and β-defensins. In normal skin these peptides are negligible, but they accumulate in skin affected by inflammatory diseases such as psoriasis. We compared the levels of expression of LL-37 and human β-defensin 2 (HBD-2) in inflamed skin from patients with atopic dermatitis and from those with psoriasis. *Methods* The expression of LL-37 and HBD-2 protein in skin-biopsy specimens from patients with psoriasis, patients with atopic dermatitis, and normal subjects was determined by immunohistochemical analysis. The amount of antimicrobial peptides in extracts of skin samples was also analyzed by immunodot blot analysis (for LL-37) and Western blot analysis (for HBD-2). Quantitative, real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays were used to confirm the relative expression of HBD-2 and LL-37 messenger RNA (mRNA) in the skin-biopsy specimens. These peptides were also tested for antimicrobial activity against *Staphylococcus aureus* with the use of a colony-forming assay. *Results* Immunohistochemical analysis confirmed the presence of abundant LL-37 and HBD-2 in the superficial epidermis of all patients with psoriasis. In comparison, immunostaining for these peptides was significantly decreased in acute and chronic lesions from patients with atopic dermatitis (P=0.006 and P=0.03, respectively). These results were confirmed by immunodot blot and Western blot analyses. Real-time RT-PCR showed significantly lower expression of HBD-2 mRNA and LL-37 mRNA in atopic lesions than in psoriatic lesions (P=0.009 and P=0.02, respectively). The combination of LL-37 and HBD-2 showed synergistic antimicrobial activity by effectively killing *S. aureus*. *Conclusions* A deficiency in the expression of antimicrobial peptides may account for the susceptibility of patients with atopic dermatitis to skin infection with *S. aureus*.

2. Immunogenetics – Gene rearrangements in the generation of immune system diversity

LANDMARK PUBLICATION:

Jerne NK
The somatic generation of immune regulation
Eur J Immunology 1971;1:1-9

3. Gell and Coombs Classification of Immune Responses

LANDMARK PUBLICATION:
Coca AF, Cooke RA
On the classification of the phenomenon of hypersensitiveness.
J Immunol 1923;8:163-182

4. T cell mediated immunity

a. T cell mediated immune responses – participating cells. Properties and functions of antigen presenting cells.

LANDMARK PUBLICATION:
Cher DJ, Mosmann TR.
Two types of murine helper T cell clone. II. Delayed-Type Hypersensitivity is Mediated by TH1 Clones.
J Immunology 1987;138:3688-94.

We have previously shown that at least two types of Lyt-1+, Lyt-2-, L3T4+ helper T cell clones can be distinguished in vitro by different patterns of lymphokine secretion and by different forms of B cell help. Evidence is presented here to show that one type of helper T cell clone (TH1) causes delayed-type hypersensitivity (DTH) when injected with the appropriate antigen into the footpads of naive mice. The antigen-specific, major histocompatability complex (MHC)-restricted footpad swelling reaction peaked at approximately 24 hr. Footpad swelling was induced by all TH1 clones tested so far, including clones specific for soluble, particulate, or allogeneic antigens. In contrast, local transfer of TH2 cells and antigen did not produce a DTH reaction, even when supplemented with syngeneic spleen accessory cells. Similarly, local transfer of an alloreactive cytotoxic T lymphocyte clone into appropriate recipients did not produce DTH. The requirements for the DTH reaction induced by TH1 cells were investigated further by using TH1 clones with dual specificity for both foreign antigens and M1s antigens. Although these clones responded in vitro to either antigen + syngeneic presenting cells, or M1s disparate spleen cells, they responded in vivo only to antigen + MHC and did not cause footpad swelling in an M1s-disparate mouse in the absence of antigen. Moreover, in vitro preactivation of TH1 or TH2 cells with the lectin concanavalin A was insufficient to induce DTH reactions upon subsequent injection into footpads. From these results, we conclude that the lack of DTH given by TH2 clones in vivo could be due to the inability of the TH2 cells to produce the correct mediators of DTH, or to a lack of stimulation of TH2 clones in the footpad environment.

LANDMARK PUBLICATION:
Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR.
Two types of murine helper T cell clone. III. Further Differences in Lymphokine Synthesis between Th1 and Th2 Clones Revealed by RNA Hybridization, Functionally Monospecific Bioassays, and Monoclonal Antibodies.
J Exp Med 1987;166:1229-44.

Lymphokine synthesis patterns of a panel of 19 T cell clones have been evaluated, using mRNA hybridization methods to examine 11 different mRNAs induced by Con A. The two types of CD4+ Th cell clone described previously were clearly distinguished by this procedure, and the differences between the two types have now been extended to six induced products. With minor exceptions, only Th1 clones synthesized mRNA for IL-2, IFN-gamma, and lymphotoxin, and only Th2 clones synthesized mRNA for IL-4, IL-5, and another induced gene, P600. Four more induced products were

expressed preferentially but not uniquely by one or another type of clone: mRNAs for GMCSF, TNF, and another induced, secreted product (TY5) were produced in larger amounts by Th1 clones, whereas preproenkephalin was preferentially expressed by Th2 clones. IL-3 was produced in similar amounts by both types of clone. mAbs were used to establish three bioassays that were functionally monospecific for IL-2, IL-3, and IL-4, and a new anti-IFN gamma mAb, XMG1.2, was used to establish an ELISA for IFN-gamma. These four assays were used to show that secreted protein and mRNA levels correlated well for all cell lines. The implications of these findings for normal T cells are discussed.

LANDMARK PUBLICATION:
Fiorentino DF, Bond MW, Mosmann TR
Two types of murine helper T cell clone. IV. Th2 Clones Secrete a Factor that Inhibits Cytokine Production by Th1 Clones..
J Exp Med 1989;170:2081-95.
A cytokine synthesis inhibitory factor (CSIF) is secreted by Th2 clones in response to Con A or antigen stimulation, but is absent in supernatants from Con A-induced Th1 clones. CSIF can inhibit the production of IL-2, IL-3, lymphotoxin (LT)/TNF, IFNgamma, and granulocyte-macrophage CSF (GM-CSF) by Th1 cells responding to antigen and APC, but Th2 cytokine synthesis is not significantly affected. Transforming growth factor beta (TGF-beta) also inhibits IFN-gamma production, although less effectively than CSIF, whereas IL-2 and IL-4 partially antagonize the activity of CSIF. CSIF inhibition of cytokine synthesis is not complete, since early cytokine synthesis (before 8 h) is not significantly affected, whereas later synthesis is strongly inhibited. In the presence of CSIF, IFN-gamma mRNA levels are reduced slightly at 8, and strongly at 12 h after stimulation. Inhibition of cytokine expression by CSIF is not due to a general reduction in Th1 cell viability, since actin mRNA levels were not reduced, and proliferation of antigen-stimulated cells in response to IL-2, was unaffected. Biochemical characterization, mAbs, and recombinant or purified cytokines showed that CSIF is distinct from IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IFN-gamma, GM-CSF, TGF-beta, TNF, LT, and P40. The potential role of CSIF in crossregulation of Th1 and Th2 responses is discussed.

5. B cell mediated immunity

a. Maturation of the antibody response

LANDMARK PUBLICATION:
Muramatsu M
Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme
Cell 2000;102:541-4

LANDMARK PUBLICATION:
Burnet, F. M.
A modification of Jerne’s theory of antibody production using the concept of clonal selection.
Australian Journal of Science. 1957: 20, 67-69.

b. Biologic process initiated by antibody: opsonization, complement fixation, antibody dependent cell mediated cytotoxicity

LANDMARK INVESTIGATION:
Graziano RF
Fc gamma RI and Fc gamma RII on monocytes and granulocytes are cytotoxic trigger molecules for tumor cells
J Immunol. 1987;139:3536-41

As part of an effort to define the cytotoxic trigger molecules on human myeloid cells, the ability of the different Fc receptors for IgG (Fc gamma R) to mediate killing of tumor cell lines by monocytes and granulocytes was examined. This was accomplished by studying cytolysis of hybridoma cell (HC) targets bearing surface antibody directed toward the different Fc gamma R. The HC line, HC IV.3A, which bears Ig directed to the low affinity Fc gamma R (Fc gamma RII) on monocytes and neutrophils was lysed by human monocytes. The extent of lysis of HC IV.3A was approximately equal to that of anti-Fc gamma RI (the high affinity Fc gamma R on human monocytes) bearing HC lines (HC 32.2A and HC 62A) and was not augmented by treatment of the monocytes with interferon-gamma (IFN-gamma). In contrast, neutrophils lysed HC IV.3A and HC 32.2A only after activation with IFN-gamma. Since Fc gamma RI is not detectable on untreated neutrophils and is induced by IFN-gamma on these cells, lysis of HC 32.2A by IFN-gamma-activated neutrophils correlated with receptor induction. On the other hand, Fc gamma RII was present at equal levels on untreated and IFN-gamma-treated neutrophils, but only IFN-gamma-treated neutrophils mediated cytotoxicity via Fc gamma RII. In this case, enhanced killing appeared to be due to events other than an increase in Fc gamma RII number. Neither untreated nor IFN-gamma-treated neutrophils mediated the lysis of the anti-Fc gamma RIII bearing HC 3G8A. Thus, binding to the tumor target via this Fc receptor does not lead to lysis and may initiate signals distinct from those triggered through Fc gamma RI or Fc gamma RII. Surprisingly, HC bearing high amounts of mouse IgG1 antibody of irrelevant specificity were also lysed by monocytes. This lysis was blocked by soluble IV.3 antibody and thus appeared to be due to binding of the Fc portion of the surface Ig to Fc gamma RII on monocytes. Furthermore, monocytes from donors with a form of Fc gamma RII incapable of binding aggregated mouse IgG1 did not lyse these HC, but displayed normal lysis of HC IV.3, demonstrating that this structurally different Fc gamma RII remained a functional trigger molecule. Overall, these studies have demonstrated the specificity of Fc receptors in triggering monocyte- and granulocyte-mediated antibody-dependent tumor cell killing and have begun to dissect functional similarities and differences among the three defined Fc gamma R on human myeloid cells.

c. IgE mediated immediate and late phase reactions

LANDMARK PUBLICATION:

Solley GO

The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies

J Clin Invest. 1976;58:408-20

IgE antibodies are usually thought to induce only immediate skin reactions. We have shown that the intradermal injection of a number of different allergens can produce a prolonged inflammatory reaction after the immediate wheal and flare in most sensitive subjects. This late inflammatory response occurs 6-12 h after challenge and is characterized by diffuse edema, erythema, pruritus, and heat. Both immediate and late responses can also be seen after passive sensitization of skin sites in nonatopic subjects. That IgE is involved in inducing the reaction was shown by the abolition of both immediate and late responses by passive transfer tests in the following experiments: (a) heating atopic serum at 56degreesC for 4 h, (b) removing IgE from the atopic serum by a solid phase anti-IgE immunoabsorbent, and (c) competitively inhibiting the binding of IgE antibodies to cells by an IgE myeloma protein. In addition, both responses were induced by affinity chromatography-purified IgE antibody, followed by antigenic challenge. Very similar lesions could also be induced by intradermal injection of Compound 48/80, thus suggesting a central role in the reaction for the mast cell or basophil. Histologically, the late phase is characterized by edema and a mixed cellular infiltration, predominantly lymphocytic but also containing eosinophils, neutrophils and basophils. Direct immunofluorescent staining did not show deposition of immunoglobulins or complement components, except IgM in 2 of 15 and C3 in 1 of 15 patients. This finding indicates that the late phase does not depend on the deposition of immune complexes. The results of the study suggest that IgE-allergen interaction on the surfaces of mast cells or on infiltrating basophils causes both immediate and late cutaneous responses.

6. Other immune and inflammatory mechanisms

a. Lymphokine activated killer cells and their effects

LANDMARK PUBLICATION:

Grimm EA

Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes.

J Exp Med. 1982;155:1823-41

Activation in lectin-free interleukin 2 (IL-2) containing supernatants of peripheral blood mononuclear leukocytes (PBL) from cancer patients or normal individuals resulted in expression of cytotoxicity toward 20 of 21 natural killer (NK)-resistant fresh solid tumor cells tested. Fresh solid tumor cells were resistant to NK-mediated lysis in 10 autologous patients' PBL-tumor interactions, and from 17 normal individuals tested against 13 allogeneic fresh tumors. Culture of PBL in IL-2 for 2-3 d was required for the lymphokine activated killers (LAK) to be expressed, and lytic activity toward a variety of NK-resistant fresh and cultured tumor targets developed in parallel. Autologous IL-2 was functional in LAK activation, as well as interferon-depleted IL-2 preparations. Irradiation of responder PBL before culture in IL-2 prevented LAK development. Precursors of LAK were present in PBL depleted of adherent cells and in NK-void thoracic duct lymphocytes, suggesting that the precursor is neither a monocyte nor an NK cell. LAK effectors expressed the serologically defined T cell markers of OKT.3, Leu-1, and 4F2, but did not express the monocyte/NK marker OKM-1. Lysis of autologous fresh solid tumors by LAK from cancer patients' PBL was demonstrated in 85% of the patient-fresh tumor combinations. Our data present evidence that the LAK system is a phenomenon distinct from either NK or CTL systems that probably accounts for a large number of reported nonclassical cytotoxicities. The biological role of LAK cells is not yet known, although it is suggested that these cells may be functional in immune surveillance against human solid tumors.

B. Immunoregulatory Mechanisms

1. Tolerance

LANDMARK ARTICLE:

Aluvihare VR, Kallikourdis M, Betz AG

Regulatory T cells mediate maternal tolerance to the fetus

Nat Immunol. 2004 Mar;5(3):266-71.

Pregnancy constitutes a major challenge to the maternal immune system, as it has to tolerate the persistence of paternal alloantigen. Although localized mechanisms contribute to fetal evasion from immune attack, maternal alloreactive lymphocytes persist. We demonstrate here an alloantigenin dependent, systemic expansion of the maternal CD25+ T cell pool during pregnancy and show that this population contains dominant regulatory T cell activity. In addition to their function in suppressing autoimmune responses, maternal regulatory T cells suppressed an aggressive allogeneic response directed against the fetus. Their absence led to a failure of gestation due to immunological rejection of the fetus.

2. Apoptosis

LANDMARK ARTICLE:

Li Yu, Ajjai Alva, Helen Su, Parmesh Dutt et al.Regulation of an ATG7-beclin 1 Program of Autophagic Cell Death by Caspase-8

Science June 2004;304: 1500 – 1502

Caspases play a central role in apoptosis, a well-studied pathway of programmed cell death. Other programs of death potentially involving necrosis and autophagy may exist, but their relation to apoptosis and mechanisms of regulation remains unclear. We define a new molecular pathway in which activation of the receptor-interacting protein (a serine-threonine kinase) and Jun aminoterminal kinase induced cell

death with the morphology of autophagy. Autophagic death required the genes *ATG7* and *beclin 1* and was induced by caspase-8 inhibition. Clinical therapies involving caspase inhibitors may arrest apoptosis but also have the unanticipated effect of promoting autophagic cell death.

3. Anergy

LANDMARK ARTICLE

Boussiotis VA, Freeman GJ , Berezovskaya A, et al.

Maintenance of Human T Cell Anergy: Blocking of IL-2 Gene Transcription by Activated Rap1

Science 1997;278:124-8}

In the absence of costimulation, T cells activated through their antigen receptor become unresponsive (anergic) and do not transcribe the gene encoding interleukin-2 (IL-2) when restimulated with antigen. Anergic alloantigen-specific human T cells contained phosphorylated Cbl that coimmunoprecipitated with Fyn. The adapter protein CrkL was associated with both phosphorylated Cbl and the guanidine nucleotide-releasing factor C3G, which catalyzes guanosine triphosphate (GTP) exchange on Rap1. Active Rap1 (GTP-bound form) was present in anergic cells. Forced expression of low amounts ofRap1-GTP in Jurkat T cells recapitulated the anergic defect and blocked T cell antigen receptor (TCR)-and CD28-mediated IL-2 gene transcription. Therefore, Rap1 functions as a negative regulator of TCRmediated IL-2 gene transcription and may be responsible for the specific defect in IL-2 production in T cell anergy.

C. Laboratory Measurements

1. Methodology and interpretation: measurements of immunoglobulin levels, immunoglobulin classes and subclasses

a. Serologic testing

i. RAST Inhibition techniques

LANDMARK ARTICLE:

Gleich GJ, Leiferman KM, Jones RT, et al.

Analysis of the potency of extracts of June grass pollen by their inhibitor capacities in the radioallergosorbent test

J Allergy Clin Immunol. 1976;58:31-8.

The potencies of 11 commercial extracts of June grass pollen were analyzed by skin test end pointtitrations and compared to potencies as determined in vitro (1) by the radioallergosorbent test (RAST),(2) by Group I antigen content, and (3) by protein nitrogen units (PNU). RAST potencies were determined by the capacity of the extract to inhibit the binding of IgE antibody to solid-phase allergen, and they were expressed as the quantity of extract required for 50% inhibition of binding. Potencies determined by skin testing in 8 patients were significantly related among the various patients in 19 of 27 comparisons and showed differences of up to 95,000-fold in the strengths of the extracts. Estimation of potencies by RAST inhibition showed approximately a 100-fold difference among the extracts and in 5 of 8 cases these were significantly related to potencies measured by skin tests. Similarly, PNU determinations and Group I determinations were also significantly related to potencies by skin test titration in 5 of 8 and in 4 of 8 comparisons, respectively. Comparison of the geometric mean skin test potencies with RAST, PNU, and Group I potencies revealed that all were significantly related to skin test potencies although the correlation of RAST and skin potency was the highest. The results indicate that measurement of potency by RAST inhibition compares favorably with other in vitro measurements of potency. These results are compared with those of a prior study with extracts of short ragweed, and the reasons for the differences between the results in the two studies are discussed.

b. Genetic techniques including TRECs, PCR and use of probes.

LANDMARK ARTICLE TREC:
Livak, F. & Schatz, D.
T-cell receptor α locus V(D)J recombination by-products are abundant in thymocytes and mature T cells. Mol. Cell. Biol: 1996;16:609-618.
In addition to the assembled coding regions of immunoglobulin and T-cell receptor (TCR) genes, the V(D)J recombination reaction can in principle generate three types of by-products in normal developing lymphocytes: broken DNA molecules that terminate in a recombination signal sequence or a coding region (termed signal or coding end molecules, respectively) and DNA molecules containing fused recombination signal sequences (termed reciprocal products). Using a quantitative Southern blot analysis of the murine TCR alpha locus, we demonstrate that substantial amounts of signal end molecules and reciprocal products, but not coding end molecules, exist in thymocytes, while peripheral T cells contain substantial amounts of reciprocal products. At the 5' end of the J alpha locus, 20% of thymus DNA exists as signal end molecules. An additional 30 to 40% of the TCR alpha/delta locus exists as remarkably stable reciprocal products throughout Tcell development, with the consequence that the TCR C delta region is substantially retained in alpha beta committed T cells. The disappearance of the broken DNA molecules occurs in the same developmental transition as termination of expression of the recombination activating genes, RAG-1 and RAG-2. These findings raise important questions concerning the mechanism of V(D)J recombination and the maintenance of genome integrity during lymphoid development.

LANDMARK ARTICLE – TREC:
Douek DC, McFarland RD, Keiser PH, et al.
Changes in thymic function with age and during the treatment of HIV infection.
Nature 1998;396:690–695.
The thymus represents the major site of the production and generation of T cells expressing alphabeta-type T-cell antigen receptors. Age-related involution may affect the ability of the thymus to reconstitute T cells expressing CD4 cell-surface antigens that are lost during HIV infection; this effect has been seen after chemotherapy and bone-marrow transplantation. Adult HIV-infected patients treated with highly active antiretroviral therapy (HAART) show a progressive increase in their number of naive CD4-positive T cells. These cells could arise through expansion of existing naive T cells in the periphery or through thymic production of new naive T cells. Here we quantify thymic output by measuring the excisional DNA products of TCR-gene rearrangement. We find that, although thymic function declines with age, substantial output is maintained into late adulthood. HIV infection leads to a decrease in thymic function that can be measured in the peripheral blood and lymphoid tissues. In adults treated with HAART, there is a rapid and sustained increase in thymic output in most subjects. These results indicate that the adult thymus can contribute to immune reconstitution following HAART.

c. Hybridoma and monoclonal antibody technology

LANDMARK Hybridoma technology:
Köhler G, Milstein C.
Continuous cultures of fused cells secreting antibody of predefined specificity.
Nature 1975;256:495-497.

II. Anatomy and Physiology

A. Normal anatomy and physiology

1. Lower airway

LANDMARK ARTICLE:
Magnussen JS. Chicco P. Palmer AW. Van der Wall H. Vu DH.
Creation of a three-dimensional model of human segmental lung anatomy
AJR. American Journal of Roentgenology. 174(5):1333-6, 2000

OBJECTIVE: The investigation of pulmonary embolism using scintigraphic tomography requires a model of the internal architecture of the segments and subsegments in the human lung. Such a model has been developed by the segmentation and subsegmentation of an existing whole-body tissue-segmented phantom. MATERIALS AND METHODS: By using information from suitably windowed human axial CT scans, combined with the information gained from the injection of color-coded dyes into the segmental bronchi of human cadaveric lungs, the lobar and segmental boundaries were added to the existing phantom. Further refinements were added from reports in the literature regarding the predominant pattern of subsegmental bronchi in a series of human cadavers, enabling the creation of subsegmental boundaries. RESULTS: A digitized model of the segmental and subsegmental anatomy of the human lung was successfully created. External, or pleural, projections of the complex internal arrangement of the segments closely corresponded with the projections of the best available authorities on the subject. CONCLUSION: The model provides the opportunity to address several issues germane to scintigraphy and important for diagnosing pulmonary embolic disease. In particular, the model allows the manipulation of three-dimensional data sets to explore issues of importance to tomographic lung scanning.

B. Pathology of primary atopic disorders

1. Rhinitis and rhinosinusitis

a. Allergic

LANDMARK PAPER:
Meltzer EO, Hamilos DL, Hadley JA, et al
Rhinosinusitis: Establishing definitions for clinical research and patient care
J Allergy Clin Immunol 2004;114:155-212)

BACKGROUND: There is a need for more research on all forms of rhinosinusitis. Progress in this area has been hampered by a lack of consensus definitions and the limited number of published clinical trials. OBJECTIVES: To develop consensus definitions for rhinosinusitis and outline strategies useful in clinical trials. METHODS: Five national societies, The American Academy of Allergy, Asthma and Immunology; The American Academy of Otolaryngic Allergy; The American Academy of Otolaryngology Head and Neck Surgery; The American College of Allergy, Asthma and Immunology; and the American Rhinologic Society formed an expert panel from multiple disciplines. Over two days, the panel developed definitions for rhinosinusitis and outlined strategies for design of clinical trials. RESULTS: Committee members agreed to adopt the term “rhinosinusitis” and reached consensus on definitions and strategies for clinical research on acute presumed bacterial rhinosinusitis, chronic rhinosinusitis without polyposis, chronic rhinosinusitis with polyposis, and classic allergic fungal rhinosinusitis. Symptom and objective criteria, measures for monitoring research progress, and use of symptom scoring tools, quality-of-life instruments, radiologic studies, and rhinoscopic assessment were outlined for each condition. Conclusion -The recommendations from this conference should improve accuracy of clinical diagnosis and serve as a starting point for design of rhinosinusitis clinical trials

2. Early and late responses to allergen challenge:

a. Nasal Challenge:

LANDMARK PUBLICATION:

Raphael GD, Igarashi Y, White MV, Kaliner MA.

The pathophysiology of rhinitis. Sources of protein in allergen-induced nasal secretions.

J Allergy Clin Immunol. 1991;88:33-42.

Allergic rhinitis is characterized by a profuse rhinorrhea in addition to paroxysms of sneezing, nasal congestion, and pruritus. To define better the sources of nasal secretion produced during rhinitis, nasal allergen challenges were performed on nine atopic subjects with seasonal rhinitis. A single dose of allergen was sprayed into one side of the nose, and nasal lavages were collected bilaterally for 7 hours. Nasal lavages were assayed for protein (total protein, albumin, lactoferrin, and lysozyme) and mediator (histamine and prostaglandin D2) content. Protein concentrations increased and remained elevated above baseline levels in both ipsilateral and contralateral secretions for up to 3 hours after allergen challenge. The proportion of albumin relative to total protein (the albumin percent) increased on the ipsilateral side, whereas the relative proportions of lactoferrin and lysozyme (the lactoferrin percent and lysozyme percent) increased on the contralateral side. Prostaglandin D2, but not histamine, increased selectively on the ipsilateral side. These data suggest that the ipsilateral protein secretory response is due to allergen-induced mast cell mediator release causing increased vascular permeability, whereas the contralateral protein secretory response is primarily a reflex-induced glandular secretion.

b. Bronchial Challenge:

LANDMARK PUBLICATION:

Cockcroft DW, Murdock KY, Kirby J, Hargreave F.

Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine.

Am Rev Respir Dis. 1987;135:264-7

Previous data have indicated that airway responsiveness to allergen, expressed as the provocation concentration causing a 20% FEV1 fall (PC20), was dependent on nonallergic airway responsiveness (histamine PC20) and sensitivity to allergen (skin sensitivity or end-point titration). From retrospective data in 24 subjects, we developed a formula to predict allergen PC20 and examined its accuracy prospectively in 26 new subjects undergoing allergen inhalation test with doubling allergen concentrations. Allergen PC20 (APC20) was predicted from histamine PC20 (HPC20) and skin sensitivity (SS) by the formula: Log10 (APC20) = 0.69 Log10 (HPC20 X SS) + 0.11 (r = 0.85). Allergen PC20 was accurately predicted in 6, and overestimated or underestimated by 1 doubling concentration in 11, by 2 concentrations in 6, by 3 concentrations in 3, and by greater than 3 concentrations in none. From the total of 50 subjects, a new relationship was developed: Log10 (APC10) = 0.68 log10 (HPC20 X SS) (r = 0.82) from which 46 of 50 (92%) of allergen PC20 values fall within 2 doubling concentrations of the regression line (and all within 3). Early airway responsiveness to a given allergen can be predicted within a +/- 8-fold range, which is better than some investigator's test reproducibility of +/- 1 log (10-fold). Allergen inhalation tests to determine early asthmatic responsiveness to different IgE-mediated allergens can probably be replaced by the simpler and safer determinations of allergen sensitivity (SS, RAST) and histamine or methacholine airway responsiveness.

LANDMARK PUBLICATION:

Killian D, Cockcroft DW, Hargreave FE, Dolovich J.

Factors in allergen induced asthma: Relevance of the intensity of the airways allergic reactionand non-specific bronchial reactivity.

Clin Allergy 1976; 6:219–225.

Early asthmatic responses (EAR) of similar severity were produced by allergen inhalation challenges in nine asthmatic subjects. The severity of the airways allergic reaction was estimated by measuring the skin test weal size produced by the same dilution of allergen which caused the EAR. The non-specific bronchial reactivity was assessed by inhalation of incresing concentrations of histamine acid phosphate. Possible relationships between the severtiy of the airways allergic reaction, the level of non-specific bronchial hyper-reactivity and the pattern of asthmatic response were examined. There was a marked inverse correlation between the required severity of the airways allergic reaction and the non-specific bronchial reactivity (log10) of the individual (r = -0-96, P less than 0-001). The EAR was followed by a late asthmatic response (LAR) in five subjects. There was no evident correlation between the magnitude of the EAR and that of the LAR. In addition, no correlation was obtained between the pattern of response interms of EAR or LAR and the severity of the allergic reaction, or the level of non-specific bronchial reactivity. These results indicate that the allergic reaction and the non-specific bronchial reactivity are interrelated in the production of allergen-induced asthma. Thus a mild allergic reaction will induce and EAR in patients with markedly increased non-specific bronchial reactivity, whereas a severe allergic reaction is required to produce an EAR in those with only slightly increased non-specific reactivity. The lack of correlation between the occurrence of the LAR and the intensity of the airways allergic reaction, the non-specific bronchial reactivity and the intensity of the EAR indicates that other factors are involved in the development of LAR.

III. Pharmacology

A. Allergenic Proteins and Extracts for Diagnosis and Treatment

1. Allergen Extract Preparation and Standardization Methods

LANDMARK ARTICLE:
Turkeltaub PC
A standardized quantitative skin-test assay of allergen potency and stability: studies on the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results.
J Allergy Clin Immunol 1982; 70(5): 343-52
This classic article describes the methodology which is the current basis for standardizing allergen extracts.

IV. Research Principles

A. Research Ethics

LANDMARK DOCUMENT:
Declaration of Helsinki: ethical principles for medical research involving human subjects.
Available at: http://www.wma.net/e/policy/b3.htm.

NOTE:
There are several websites at the National Institutes of health that may be helpful in designing learning activities for fellows in training that are related to research ethics including…
http://ohsr.od.nih.gov/guidelines/graybook.html#app3
Guidelines for the conduct of research involving human subjects at the National Institutes of Health

http://ohsr.od.nih.gov/guidelines/belmont.html
The Belmont Report: Ethical Principles and Guidelines for the protection of human subjects of research

V. Clinical Sciences

A. Allergic Diseases and Related Disorders

1. Upper airway disease

a. Clinical skills and interpretive strategies for diagnosis of upper airway diseases: skin testing (epicutaneous and intracutaneous); cytology of nasal secretions; understanding of indications for and methodology of nasal challenges; rhinoscopy; nasal and ear examination; gross assessment of upper airway imaging studies.

i. Skin testing

LANDMARK ARTICLE:
Malling HJ
Proposed guidelines for quantitative skin prick test procedure to determine the biological activity of allergenic extracts using parallel line assay,
Allergy; 1987;42, 391-4
Guidelines are proposed for determining the potency of allergenic extracts in relation to a reference extract using parallel line bio-assay. The practical performance, limitations, and advantages of skinprick test are discussed.

2. Lower respiratory tract disease

a. Specific skills and practical management : chest exam, interpretation of pulmonary function testing, bronchial challenges, sputum and exhaled breath analysis, and gross interpretation of imaging studies.

i. LongActing beta agonists

LANDMARK ARTICLE:
Nelson HS
The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol.
Chest. 2006;129:15-26
STUDY OBJECTIVE: To compare the safety of salmeterol xinafoate or placebo added to usual asthma care. DESIGN: A 28-week, randomized, double-blind, placebo-controlled, observational study. SETTING: Study subjects were seen once in the study physician's office for screening and were provided all blinded study medication for the entire study period. Follow-up by telephone was scheduled every 4 weeks. PARTICIPANTS: Subjects (> 12 years old) with asthma as judged by the study physician were eligible. Individuals with a history of long-acting beta2-agonist use were excluded. INTERVENTIONS: Salmeterol, 42 mug bid via metered-dose inhaler (MDI), and placebo bid via MDI. MEASUREMENTS AND RESULTS: Following an interim analysis in 26,355 subjects, the study was terminated due to findings in African Americans and difficulties in enrollment. The occurrence of the primary outcome, respiratory-related deaths, or life-threatening experiences was low and not significantly different for salmeterol vs placebo (50 vs 36; relative risk [RR] = 1.40; 95% confidence interval [CI], 0.91 to 2.14). There was a small, significant increase in respiratory-related deaths (24 vs 11; RR, 2.16; 95% CI, 1.06 to 4.41) and asthma-related deaths (13 vs 3; RR, 4.37; 95% CI, 1.25 to 15.34), and in combined asthma-related deaths or life-threatening experiences (37 vs 22; RR, 1.71; 95% CI, 1.01 to 2.89) in subjects receiving salmeterol vs placebo. The imbalance occurred largely in the

African-American subpopulation: respiratory-related deaths or life-threatening experiences (20 vs 5; RR, 4.10; 95% CI, 1.54 to 10.90) and combined asthma-related deaths or life-threatening experiences (19 vs 4; RR, 4.92; 95% CI, 1.68 to 14.45) in subjects receiving salmeterol vs placebo. CONCLUSIONS: For the primary end point in the total population, there were no significant differences between treatments. There were small, but statistically significant increases in respiratory-related and asthma-related deaths and combined asthma-related deaths or life-threatening experiences in the total population receiving salmeterol. Subgroup analyses suggest the risk may be greater in African Americans compared with Caucasian subjects. Whether this risk is due to factors including but not limited to a physiologic treatment effect, genetic factors, or patient behaviors leading to poor outcomes remains unknown.

ii. Genetic polymorphisms and beta agonists

LANDMARK INVESTIGATION:
Martinez FD.
Association between genetic polymorphisms of the b2-adrenoceptor and response to albuterol in children with and without a history of wheezing.
J Clin Invest 1997;100:3184-3188.

The beta2-adrenergic receptor (beta2AR) agonists are the most widely used agents in the treatment of asthma, but the genetic determinants of responsiveness to these agents are unknown. Two polymorphic loci within the coding region of the beta2AR have been recently described at amino acids 16 and 27. It has been reported that glycine at codon 16 (Gly-16) is associated with increased agonist-promoted downregulation of the beta2AR as compared with arginine-16 (Arg-16). The form of the receptor with glutamic acid at codon 27 (Glu-27), on the other hand, has been shown to be resistant to downregulation when compared with glutamine-27 (Gln-27), but only when coexpressed with Arg-16. To assess if different genotypes of these two polymorphisms would show differential responses to inhaled beta2AR agonists, we genotyped 269 children who were participants in a longitudinal study of asthma. Spirometry was performed before and after administration of 180 microg of albuterol, and a positive response was considered an increase of >15.3% predicted FEV1. There was marked linkage disequilibrium between the two polymorphisms, with 97.8% of all chromosomes that carried Arg-16 also carrying Gln-27. When compared to homozygotes for Gly-16, homozygotes for Arg-16 were 5.3 times (95% confidence interval 1.6-17.7) and heterozygotes for beta2AR-16 were 2.3 times (1.3-4.2) more likely to respond to albuterol, respectively. Similar trends were observed for asthmatic and nonasthmatic children, and results were independent of baseline lung function, ethnic origin, and previous use of antiasthma medication. No association was found between the beta2AR-27 polymorphism and response to albuterol. These results may explain some of the variability in response to therapeutic doses of albuterol in children.

3. Drug allergy

a. General reviews and susceptibility states

LANDMARK PUBLICATION:
Brown BC, Price EV, Moore MD
Penicilloyl-polylysine as an intradermal test of penicillin sensitivity.
JAMA 1964;189:599-604.

4. Anaphylaxis and anaphylactoid reactions

LANDMARK PUBLICATION:
Smith PL, Kagey-Sobotka A, Bleecker ER, et al. Physiologic manifestations of human anaphylaxis. J Clin Invest 1980;66:1072-80.

The authors conducted a controlled study to evaluate different forms of immunotherapy for subjects with

insect-sting hypersensitivity, and observed 11 subjects who had generalized urticaria and 3 subjects who experienced anaphylactic shock characterized by severe hypotension attributed to peripheral vasodilation. Plasma histamine levels correlated with the severity and duration of the cardiopulmonary changes observed during anaphylactic shock. The two subjects with the most severe shock showed reductions in Factor V, Factor VIII, fibrinogen, and high molecular weight kininogen, as well as changes in complement components. This study also documents the paradoxical occurrence of bradycardia in the setting of anaphylactic shock (one subject).

5. Insect hypersensitivity

LANDMARK PUBLICATION:
Hunt KJ, Valentine MD, Sobotka AK et al
A controlled trial of immunotherapy in insect hypersensitivity
N Engl J Med 1978;299:157-161
Insect hypersensitivity is currently treated by immunization using whole-body extracts. We compared this regimen with immunotherapy using insect venoms or placebo in groups of 20 patients matched for history and sensitivity, as judged by venom skin test, histamine release and IgE antibody to venom. After six to 10 weeks of immunization, systemic reactions to stings occurred in seven of 12, seven of 11, and one of 18 patients treated with placebo, whole-body extract, and venom, respectively. Placebo and wholebody extract gave similar results and were significantly less effective than venom immunotherapy (P less than 0.01). The 14 patients with failure of treatment with wholebody extract and placebo were subsequently provided with venom immunotherapy; one reacted to a subsequent sting. We conclude that venom immunotherapy is clinically superior to therapy on whole-body extract or placebo.

LANDMARK PUBLICATION:
Barnard JH
Studies of 400 Hymenoptera sting deaths in the United States
J Allergy Clin Immunol 1973:52;259-64
Data from 400 cases of Hymenoptera sting deaths in. the United States have been collected. These included 100 cases seen at autopsy and the results of postmortem abnormalities and certain other correlations ore tabulated.

a. Skin prick, intradermal and in vitro testing to stinging insects

LANDMARK PUBLICATION:
Golden DBK, Marsh DG, Freidhoff LR, et al.
Natural history of Hymenoptera venom sensitivity in adults
J Allergy Clin Immunol 1997;100:760-6
Background: Epidemiologic studies of Hymenoptera venom allergy in adults show a prevalence of positive venom skin test results, RASTs of 15% to 25%, or both, but most such individuals have had no systemic reactions to stings. The clinical significance and natural history of this apparently common sensitivity is uncertain. Objective: We sought to determine the natural history of venom sensitization by observing the rate of decrease or increase in sensitivity in normal adults over 5 to 10 years. The clinical significance of these findings is related to the frequency of systemic reactions to stings during the period of observation. Methods: Serial observations were planned in 520 volunteers and randomly selected subjects. Two follow-up visits were attempted , once after 2 to 3 years and again after 5 to 9 years, to perform repeat venom skin tests and RASTs and to review any history of interim stings and their outcomes. Results: Follow-up visits were conducted with 398 subjects (375 early visits and 205 late visits). Overall, in the 398 subjects with one or more visits after a mean of 4 years, skin test responses changed from positive to negative in 44 of 98 (45%) and from negative to positive in 27 of 309 (8.7%) of the subjects. Skin test responses changed from positive to negative in 29 of 87 (33%) subjects after 2.5 years and in 43 of 54 (80%) after 6.8 years. Even when the skin test response became negative,

venom-specific IgE remained positive in 11 of 29 (38%) subjects after 2.5 years and in 13 of 43 (30%) after 6.8 years. The rate of loss of sensitivity was 12% per year, similar to retrospective estimates. Skin test sensitivity to venoms disappears more rapidly in these subjects without symptoms (half-life, 4 years) than in patients receiving venom immunotherapy (half-life, 7 years). Skin test responses changed from negative to positive in 23 of 288 (8%) subjects after 2.5 years and in 9 of 151 (6%) after 6.8 years. Insect stings caused no reaction in 120 subjects with a negative skin test response, but 17% (11 of 65) of subjects with a positive skin test response (but with a negative history) had systemic reactions when stung. There was no difference between the early and late visits in the frequency of systemic reactions reported. The risk may be higher than 17% for the specific individuals (67% after 2.5 years and 20% after 6.8 years) whose positive skin test responses persist for years. This risk is lower than that of patients with a positive history (50%) but higher than that of "normal" adults or venomtreated patients (<2%). It is still not clear whether any subset of adults with a positive skin test response but a negative history can be identified, in whom the risk of systemic sting reaction would justify venom immunotherapy even before any reaction occurs. Conclusion: Asymptomatic venom sensitization in adults is common but transient, disappearing at the rate of 12% per year. However, the risk of a systemic reaction to a subsequent sting is significant in adults without symptoms but with positive venom skin test responses (17%) and may be higher when skin test sensitivity does persist for years.

b. Predictive value of clinical history and testing for adult and pediatric population

LANDMARK PUBLICATION:

Outcomes of Allergy to Insect Stings in Children, with and without Venom Immunotherapy
Golden DBK, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM
N Engl J Med 2004:351;668-74.

Background - Children are thought to “outgrow” the allergy to insect stings, but there are no reports documenting the natural history of this reaction. We studied the outcome of allergic reactions to insect stings in childhood 10 to 20 years afterward in patients who had not received venom immunotherapy and in those who had been treated. Methods - Between 1978 and 1985, we diagnosed allergic reaction to insect stings in 1033 children, of whom 356 received venom immunotherapy. We conducted a survey of these patients by telephone and mail between January 1997 and January 2000, to determine the outcome of stings that occurred in the period from 1987 through 1999. Results - Of the 1033 patients, 512 patients (50 percent) responded, with a mean follow-up period of 18 years, a mean duration of venom immunotherapy of 3.5 years in treated patients, and an incidence of stings of 43 percent. Systemic reactions occurred less frequently in patients who had received venom immunotherapy (2 of 64 patients, or 3 percent) than in untreated patients (19 of 111 patients, or 17 percent; P=0.007). Patients - with a history of moderate-to-severe reactions had a higher rate of reaction if they had not been treated (7 of 22 patients, or 32 percent) than if they had received venom immunotherapy (2 of 43 patients, or 5 percent; P=0.007). In patients who had been treated and who had a history of mild (cutaneous) systemic reaction (i.e., one with only cutaneous manifestations), none of the 21 subjects who received stings had a systemic reaction. Conclusions - A clinically important number of children do not outgrow allergic reactions to insect stings. Venom immunotherapy in children leads to a significantly lower risk of systemic reaction to stings even 10 to 20 years after treatment is stopped, and this prolonged benefit is greater than the benefit seen in adults.

c. Venoms, formulation, schedule and duration of immunotherapy.

LANDMARK PUBLICATION:

Freeman TM, Hylander R, Ortiz A, Martin ME
Imported fire ant immunotherapy:m Effectiveness of whole body extracts
J Allergy Clin Immunol 1992:90;210-5

The purpose of this study was to determine if whole body extract (WBE) immunotherapy for Imported fjre ant (IFA) hypersensitivity is effective. This evaluation was carried out by retrospectively interviewing 76 patients with a history of generalized allergic reactions to IFA stings and positive skin tests to IFA-WBE. The study groups consisted of 65 patients onimmunotherapy and 11 similar patients who were not treated for various reasons. In addition, an IFA sting challenge was performed in 30 volunteers of the 65 patients on immunotherapy. The retrospective review showed that of the 65 patients on immunotherapy there had been 112 Subsequent field-sting episodes in 47 patients. Only one sting episode in this group (2.1%) produced an anaphylactic reaction. Six of the 11 patients not on immunotherapy have had subsequent field re-sting episodes, and each has had a systemic reaction. Repeat skin testing on 31 of the 65 patients in the immunotherapy group showed persistent positive responses in five (16%), but each was at a lower dilution than initially. Responses of the other 26 of the 31 patients who had skin testing had become negative. The four untreated patients who were available for skin testing continued to have positive responses at comparable dilutions on skin testing. Sting challenges carried out on 30 volunteers from the 65 patients (all from the 31 who had repeat skin tests) on immunotherapy resulted in only local reactions. Therefore it appears IFA-WBE is effective in decreasing the incidence of anaphylaxis during subsequent field stings; reducing specific immunoglobulin E as demonstrated by skin testing; and protecting against systemic reactions provoked by a sting challenge with a single IFA.

LANDMARK PUBLICATION:
Golden DBK, Kagey-Sobotka A, Lichtenstein LM
Survey of patients after discontinuing venom immunotherapy
J Allergy Clin Immunol 2000;105:385-90
Background: Venom immunotherapy rapidly reduces the risk of a systemic sting reaction in adults from 30% to 70% to less than 2%. When venom immunotherapy is stopped after 5 years or longer, the risk of a systemic sting reaction is 5% to 15% during the first few years after stopping treatment. It is uncertain whether systemic sting reactions will occur more than 5 years after discontinuing venom immunotherapy and whether treatment can be safely stopped in some patients after less than 5 years. Objective: The purpose of this study is to estimate the risk of systemic reaction to a sting 10 years after discontinuing treatment and the relative risk after 3 years of treatment compared with that after 5 years or more of treatment. Methods: Among all patients who had venom immunotherapy at our center, we identified 395 patients who stopped treatment: some had dropped out of therapy early (6-24 months), some stopped after 3 to 4 years, and most completed 5 years or more of venom immunotherapy and were advised to stop by the allergist (many as part of our reported studies of discontinuing venom immunotherapy). Results: Contact was made with 194 patients, including telephone interviews for sting history and requests to visit the office for skin testing and blood sampling. Of these patients, 74 had been included in our original study of patients who had 5 years or more of venom immunotherapy and had sting challenges after 1 to 5 years off venom immunotherapy, as previously reported. Of the 74 in that original study, 61 were reached for this survey, and 30 reported recent stings, with 5 systemic sting reactions. Another 133 patients who had stopped venom immunotherapy were reached: 82 had 5 or more years of venom immunotherapy, 20 had 3 to 4 years of venom immunotherapy, and 31 had less than 2 years of venom immunotherapy. Of 51 patients stung from this group, 27 had 5 or more years of venom immunotherapy (no systemic sting reactions), and 24 had less than 5 years of venom immunotherapy (3 systemic sting reactions). We have now observed a total of 113 patients who had 5 or more years of venom immunotherapy and were stung after stopping. Sixteen (14%) had systemic sting reactions; most were mild, but 4 were severe. Systemic sting reactions occurred in 12 (10.7%) of 112 patients stung in the first 4 years off venom immunotherapy and 5 (10%) of 50 stung more than 5 years off venom immunotherapy. In 4 of 8 patients with current systemic sting reactions, the skin test response was negative, although the venom-IgE response was positive at the previous encounter. All systemic sting reactions were similar in pattern and severity to prevenom immunotherapy reactions in the same patient.

Conclusions:We conclude that the risk of systemic sting reactions when venom immunotherapy is stopped after 5 years or longer remains in the reported range of 5% to 15% in the 5 to 10 years after stopping venom immunotherapy. This risk of systemic sting reactions does not seem to decrease over time, unlike the progressive decline in immunologic markers (skin test and venom-IgE responses). To prospectively assess the risk of recurrent systemic sting reactions, there is a need for sting challenge studies of patients who have been off venom immunotherapy for 5 to 10 years and patients who have stopped venom immunotherapy after just 3 to 4 years treatment.

LANDMARK PUBLICATION:
Ruëff F, Wenderoth A, Przybilla B
Patients still reacting to a sting challenge while receiving conventional Hymenoptera venom immunotherapy are protected by increased venom doses
J Allergy Clin Immunol 2001;108:1027-32
Background: Up to 20% of patients allergic to Hymenoptera venom are not protected by conventional venom immunotherapy (VIT) with 100 μg of any single venom. Objective:We sought to evaluate the efficacy of an increased venom dose in patients allergic to Hymenoptera venom still reacting systemically to a sting challenge despite immunotherapy with 100 μg of venom every 4 weeks. Methods: In this retrospective study patients were included who still had reacted systemically to a sting challenge with a living bee or wasp despite VIT with a maintenance dose of 100 μg every 4 weeks. The maintenance dose was increased to 150 or 200 μg every 4 weeks, and a second sting challenge was performed. If a patient reacted again, the dose was further increased. Baseline mast-cell tryptase levels were assessed by using a fluoroenzyme immunoassay in stored patient sera. Results: While receiving a maintenance dose of 100 μg of venom every 4 weeks for 7 to 38 months, 18 patients reacted systemically to a bee sting and 22 reacted to a wasp sting. After an increase of the maintenance dose to 150 μg, 2 of 4 patients allergic to bee venom (BV) and 6 of 6 patients allergic to yellow jacket venom (YJV) no longer reacted systemically to the sting challenge. The respective rates of full protection were 13 of 14 and 15 of 16 in patients with an increase of the maintenance dose to 200 μg from the start. Of those 4 individuals not protected by the first dose increase, one patient allergic to BV (prior dose of 150 μg) and one patient allergic to YJV (prior dose of 200 μg) did not react systemically to a further sting challenge while receiving 200 μg of BV or 250 μg of YJV, respectively. One patient allergic to BV who had a systemic reaction to the sting challenge while receiving 150 μg was not protected after a dose increase to 200 μg; she later received a dose of 400 μg of BV, and no further sting challenge was performed. The patient allergic to BV who still reacted systemically after a first dose increase to 200 μg was a female patient with urticaria pigmentosa. She had repeated systemic adverse reactions to further BV immunotherapy, necessitating discontinuation of the treatment; however, she tolerated well VIT with 200 μg of YJV. In all other patients, no unusual adverse reactions to the increased venom doses were observed. Baseline serum tryptase levels were elevated above 13.5 μg/L (95^{th} percentile in normal subjects) in 9 (28.1%) of 32 patients. Conclusions: The majority of patients allergic to Hymenoptera venom who still reacted systemically to a sting challenge despite VIT with a dose of 100 μg every 4 weeks can be fully protected by an increased maintenance dose. This dose increase is well tolerated by most patients. The rather high proportion of patients with elevated baseline serum tryptase levels necessitates further investigation of a possible association between mastocytosis and treatment failure of conventionally dosed VIT.

B. Transplantation Medicine

1. GVHD: acute and chronic

LANDMARK ARTICLE:
Martin PJ , Hansen JA, Buckner CD, et al.
Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts

Blood 1985;66:664-672

We report results of a pilot study designed to evaluate the effects of in vitro depletion of T lymphocytes from donor marrow in patients receiving HLA-identical marrow grafts for treatmentof hematologic malignancies. Twenty patients aged 31 to 50 years were prepared fortransplantation with cyclophosphamide (120 mg/kg) and fractionated total body irradiation (12.0or 15.75 Gy). All received cyclosporine after grafting. The donor marrows were treated with a mixture of eight murine monoclonal antibodies and rabbit serum complement in a manner that achieved a 2- to 3-log depletion of T cells in most patients. Initial engraftment occurred promptly in 19 of the patients, and only three had clinically significant acute graft-versus-host disease. Depletion of donor T cells, however, was associated with an increased incidence of graft failure, which occurred as late as 244 days after transplantation. Graft failure was transient in one patient but apparently was irreversible in seven others. Three of the seven patients had cytogenetic but not morphological evidence of leukemic relapse at the time of graft failure. All seven patients with irreversible graft failure have died, six after receiving second bone marrow transplants. Seven of the eight cases of graft failure occurred among the 11 patients prepared for transplantation with 12.0 Gy of total-body irradiation, and only one occurred among the nine patients with advanced malignancies who received 15.75 Gy of total-body irradiation. This association with irradiation dose suggests that host factors were partly responsible for the graft failures. Because graft failure seldom occurs in irradiated recipients of unmodified HLA identicalallogeneic marrow transplants, it appears that T cells in the donor marrow may serve a beneficial function in helping to maintain sustained engraftment possibly by eliminating host cells that can cause graft failure. Optimal application of in vitro manipulation of donor marrow asa method for preventing graft-versus-host disease will require more effective immunosuppression of the recipient in order to assure sustained engraftment and function of donor stem cells.

C. Immune System Related Malignancies and Cellular Disorders

1. B cell and plasma cell neoplasms

LANDMARK ARTICLE:

Miller RA, Maloney DG, Warnke R, Levy R.

Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody

N Engl J Med. 1982;306:517-22

2. T cell neoplasms

LANDMARK ARTICLE:

Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC.

Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-celllymphoma.

Proc Natl Acad Sci 1980;77:7415.

Retrovirus particles with type C morphology were found in two T-cell lymphoblastoid cell lines, HUT 102 and CTCL-3, and in fresh peripheral blood lymphocytes obtained from a patient with a cutaneous T-cell lymphoma (mycosis fungoides). The cell lines continuously produce these viruses, which are collectively referred to as HTLV, strain CR(HTLVCR). Originally, the production of virus from HUT 102 cells required induction with 5-iodo-2′-deoxyuridine, but the cell line became a constitutive producer of virus at its 56th passage. Cell line CTCL-3 has been a constitutive producer of virus from its second passage in culture. Both mature and immature extracellular virus particles were seen in thin-section electron micrographs of fixed, pelleted cellular material; on occasion, typical type C budding virus particles were seen. No form of intracellular virus particle has been seen. Mature particles were 100-110 nm in diameter, consisted of an electron-dense core surrounded by an outer membrane separated by an electronlucent region, banded at a density of 1.16 g/ml on a continuous 25-65% sucrose gradient, and contained 70S RNA and a DNA polymerase activity typical of viral reverse transcriptase

(RT; RNA-dependent DNA nucleotidyltransferase). Under certain conditions of assay, HTLVCR RT showed cation preference for Mg2+ over Mn2+, distinct from the characteristics of cellular DNA 317 polymerases purified from human lymphocytes and the RT from most type C viruses. Antibodies to cellular DNA polymerase γ and anti-bodies against RT purified from several animal retroviruses failed to detectably interact with HTLVCR RT under conditions that were positive for the respective homologous DNA polymerase, demonstrating a lack of close relationship of HTLVCR RT to cellular DNA polymerases γ or RT of these viruses. Six major proteins, with sizes of approximately 10,000, 13,000, 19,000, 24,000, 42,000, and 52,000 daltons, were apparent when doubly banded, disrupted HTLVCR particles were chromatographed on a NaDodSO4/polyacrylamide gel. The number of these particle-associated proteins is consistent with the expected proteins of a retrovirus, but the sizes of some are distinct from those of most known retroviruses of the primate subgroups.

3. Mast cell dyscrasias

LANDMARK ARTICLE:
Schwartz LB, Metcalf DD,Miller JS, et al.
Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis
N Engl J Med 1987 316:1622-1626

Better methods are needed to assess mast-cell activation in vivo and to distinguish the activation Better methods are needed to assess mast-cell activation in vivo and to distinguish the activation of mast cells from that of basophils. Tryptase, a neutral protease selectively concentrated in the secretory granules of human mast cells (but not basophils), is released by mast cells together with histamine and serves as a marker of mast-cell activation. In 17 patients with systemic mastocytosis, concentrations of tryptase in plasma were linearly related to those of histamine (P less than 0.01). Eleven of the 17 patients had tryptase levels of 4 to 88 ng per milliliter, indicating ongoing mast-cell activation. In each of six patients who experienced corresponding anaphylactic reactions after penicillin, aspirin, or melon ingestion, a wasp sting, exercise, or antilymphocyte globulin injection, tryptase levels in serum ranged from 9 to 75 ng per milliliter, indicating mast-cell activation during each of these events. In contrast, serum tryptase levels were less than 5 ng per milliliter in all patients presenting with myocardial disease (n = 8, 6 with hypotension) or sepsis (n = 6, 3 with hypotension) and in the controls (n = 20). One patient had a myocardial infarction after anaphylaxis in response to a wasp sting and an elevated tryptase level of 25 ng per milliliter. Thus, the plasma or serum tryptase level is a diagnostic correlate of mast cell related events.

4. Eosinophilic disorders

LANDMARK PAPER:
Cools J. DeAngelo DJ. Gotlib J. Stover EH. Legare RD. Cortes J. Kutok J. Clark J. Galinsky I. Griffin JD. Cross NC. Tefferi A. Malone J. Alam R. Schrier SL. Schmid J. Rose M. Vandenberghe P. Verhoef G. Boogaerts M. Wlodarska I. Kantarjian H. Marynen P. Coutre SE. Stone R. Gilliland DG.
A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome

ABSTRACT BACKGROUND: Idiopathic hypereosinophilic syndrome involves a prolonged state of eosinophilia associated with organ dysfunction. It is of unknown cause. Recent reports of responses to imatinib in patients with the syndrome suggested that an activated kinase such as ABL, platelet-derived growth factor receptor (PDGFR), or KIT, all of which are inhibited by imatinib, might be the cause. METHODS: We treated 11 patients with the hypereosinophilic syndrome with imatinib and identified the molecular basis for the response. RESULTS: Nine of the 11 patients treated with imatinib had responses lasting more than three months in which the eosinophil count returned to normal. One such patient had a complex chromosomal abnormality, leading to the identification of a fusion of the Fip1-

like 1 (FIP1L1) gene to the PDGFRalpha (PDGFRA) gene generated by an interstitial deletion on chromosome 4q12. FIP1L1-PDGFRalpha is a constitutively activated tyrosine kinase that transforms hematopoietic cells and is inhibited by imatinib (50 percent inhibitory concentration, 3.2 nM). The FIP1L1-PDGFRA fusion gene was subsequently detected in 9 of 16 patients with the syndrome and in 5 of the 9 patients with responses to imatinib that lasted more than three months. Relapse in one patient correlated with the appearance of a T674I mutation in PDGFRA that confers resistance to imatinib. CONCLUSIONS: The hypereosinophilic syndrome may result from a novel fusion tyrosine kinase - FIP1L1-PDGFRalpha - that is a consequence of an interstitial chromosomal deletion. The acquisition of a T674I resistance mutation at the time of relapse demonstrates that FIP1L1-PDGFRalpha is the target of imatinib. Our data indicate that the deletion of genetic material may result in gain-of-function fusion proteins.

LANDMARK ARTICLE:
Schrezenmeier H,Thome SD, Tewald F, et al.
Interleukin-5 is the predominant eosinophilopoietin produced by cloned T lymphocytes in hypereosinophilic syndrome.
Exp Hematol. 1993;21:358-65
oned T lymphocytes (TLC) of the CD4+CD8- phenotype established from peripheral blood of a patient with idiopathic hypereosinophilic syndrome (HES) were found to release a lineagespecific eosinophilic colony-stimulating factor (Eo-CSF). The present study was undertaken to identify the lymphokine accounting for this Eo-CSF activity. Comparison of TLC-derived Eo-CSF with recombinant human interleukin-5 (rhIL-5), recombinant human granulocytemacrophage colony-stimulating factor (rhGM-CSF) and recombinant human interleukin-3 (rhIL-3) by in vitro clonogenic assays revealed similar bioactivity of HES-derived Eo-CSF and IL-5. Neutralization studies using specific antibodies against IL-5, GM-CSF and IL-3 confirmed that IL-5 mainly accounts for the Eo-CSF activity in all 9 HES-derived TLC tested. Eosinophilic colony (CFU-Eo) formation supported by conditioned media of the TLC was significantly inhibited in all clones by addition of anti-IL-5 monoclonal antibody (MAB) to the conditioned media. Inhibition by anti-IL-5 MAB was specific and dose-dependent. In 2 of the 9 clones, anti-GM-CSF antibodies could partially neutralize the Eo-CSF activity in the conditioned media. In 4 clones, addition of a combination of anti-IL-5 MAB and anti-GM-CSF antiserum to the conditioned media reduced CFU-Eo formation significantly more than addition of anti-IL-5 MAB alone. In none of the TLC could a significant role for IL-3 in eosinophilic colony formation be shown. These results were confirmed at the mRNA level. Cytokine transcripts were detected by reverse transcription (RT) and subsequent polymerase chain reaction (PCR). Under the same experimental conditions, all HES-derived TLC, but only one third of tested TLC from healthy donors, expressed IL-5 mRNA 5 days after stimulation. In control TLC with inducible IL-5 mRNA expression, IL-5 transcripts were found for only 3 days after stimulation. In contrast, HES-derived TLC contained IL-5 mRNA at least until day 18 after restimulation. All HES clones expressed GM-CSF mRNA upon stimulation. Two HES-derived TLC were found to lack IL-3 mRNA even after stimulation. Whereas IL-5 was expressed abundantly in all HESclones, the intensity of PCR products for GM-CSF and IL-3 showed striking differences. Our in vitro results suggest that IL-5 produced by activated CD4+ T lymphocytes plays a crucial role in the induction of eosinophilia in HES. In addition, GM-CSF but not IL-3 seems to contribute partially to the increased eosinophil production in HES.

5. Cryopathies and amyloid

LANDMARK ARTICLE:
Levo Y , Gorevic PD , Kassab HJ,et al
Association between hepatitis B virus and essential mixed cryoglobulinemia
N Engl J Med 1977;296:1501-1504

In view of a high frequency of liver involvement in patients with essential mixed cryoglubulinemia, we looked for evidence for hepatitis B virus infection in 25 serum specimens and 19 cryoprecipitates obtained from 30 patients. Three of the 25 serum specimens contained Hbs Ag, and 12 had antibody. The frequency of positive results was increased to six and 11 of 19 respectively when cryoprecipitates were examined, and 14 of 19 (74 per cent) of the cryoprecipitates were positive for either HBs Ag or its antibody. Electron microscopy of four cryoprecipitates showed structures resembling the 20-nm and 27-nm spheres, tubules, as well as the Dane particles characteristic of hepatitis B virus infection. Since such infection appears to be involved in the pathogenesis of the syndrome, the term "essential mixed cryoglobulinemia" should be replaced by "mixed cryoglobulinemia secondary to hepatitis B virus" or perhaps to other viral infections.

D. Established and Evolving Immune-based Treatment Modalities

1. Glucocorticoids and immunosuppressants (also see Section III.A.)

LANDMARK ARTICLE:
Hench PS, Kendall EC, Slocumb CH, Polley HF.
Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis, rheumatic fever and certain other conditions: A study in clinical physiology.
Arch Intern Med. 1950;85:546–666.

LANDMARK ARTICLE:
Boardley JE, Carey RA, Harvey AM.
Preliminary observations on the effect of adrenocorticotropic hormone in allergic diseases.
Bull. Johns. Hopkins. Hosp. 1949; 85, 396–410.
The Nobel Prize for Medicine was awarded in 1950 to Hench for the discovery of synthesized ACTH and cortisol where it was efficaciously used in rheumatoid arthritis. This study was published around the time documenting efficacy in 5 asthmatic patients with eosinophilic sputums who improved and had resolution of sputum eosinophilia after a 3 week period of ACTH injections. It was later confirmed that oral cortisol had the same beneficial effects.

2. Nucleic acid based therapies (DNA vaccines, CpG, gene insertion, antisense nucleotides)

LANDMARK ARTICLE:
Tokunaga T, Yamamoto H, Shimada S, et al
Antitumor activity of deoxyribonucleic acid fraction from Mycobacterium bovis BCG. I. Isolation, physicochemical characterization, and antitumor activity.
J Natl Cancer Inst. 1984 Apr;72(4):955-62.
A fraction extracted from Mycobacterium bovis strain BCG, which was composed of 70.0% DNA, 28.0% RNA, 1.3% protein, 0.20% glucose, and 0.1% lipid and of no detectable amounts of cell wall components such as alpha, epsilon-diaminopimelic acid and hexosamine, was found to possess strong antitumor activity. Repeated intralesional injection of this fraction, designated MY-1, without attachment to oil or a single intralesional injection of MY-1 emulsified in mineral oil caused the IMC carcinoma of CDF1 mice and line 10 tumor of strain 2 guinea pigs to regress and/or prevented metastasis very effectively. MY-1 after digestion with RNase, which contained 97.0% single-stranded DNA with a guanine-cytosine content of 69.8%, was more effective than undigested MY-1 against IMC and line 10 tumor, while MY-1 digested with DNase, which contained 97.0% RNA, had reduced activity, suggesting that the DNA from BCG possessed strong antitumor activity under certain conditions. Details of the extraction procedures and physicochemical characterization of MY-1 were also described.

LANDMARK:

Sato Y, Roman M, Tighe H, Lee D,
Immunostimulatory DNA sequences necessary for effective intradermal gene immunization.
Science. 1996 Jul 19;273(5273):352-4.
Vaccination with naked DNA elicits cellular and humoral immune responses that have a T helper cell type 1 bias. However, plasmid vectors expressing large amounts of gene product do not necessarily induce immune responses to the encoded antigens. Instead, the immunogenicity of plasmid DNA (pDNA) requires short immunostimulatory DNA sequences (ISS) that contain a CpG dinucleotide in a particular base context. Human monocytes transfected with pDNA or double-stranded oligonucleotides containing the ISS, but not those transfected with ISS-deficient pDNA or oligonucleotides, transcribed large amounts of interferon-alpha, interferon-beta, and interleukin-12. Although ISS are necessary for gene vaccination, they down-regulate gene expression and thus may interfere with gene replacement therapy by inducing proinflammatory cytokines.

3. Cytokine receptors and receptor antagonists (IFN, antiTNF, etc)

LANDMARK PUBLICATION:
Strander H, Cantell K et al.
Clinical and laboratory investigations on man: systemic administration of potent interferon to man.
J Natl Cancer Inst 1973 Sep; 51(3):733-42.

4. Probiotics

LANDMARK ARTICLE:
Majmaa H and Isolauri E.
Probiotics: A Novel Approach in the Management of Food Allergy.
J Allergy Clin Immunol. 1997 Feb; 99(2): 179-85.
BACKGROUND: The gastrointestinal microflora is an important constituent of the gut mucosal defense barrier. We have previously shown that a human intestinal floral strain, Lactobacillus GG (ATCC 53103), promotes local antigen-specific immune responses (particularly in the IgA class), prevents permeability defects, and confers controlled antigen absorption. OBJECTIVE: The aim of this study was to evaluate the clinical and immunologic effects of cow's milk elimination without (n = 14) and with (n = 13) the addition of Lactobacillus GG (5 x 10(8) colony-forming units/gm formula) in an extensively hydrolyzed whey formula in infants with atopic eczema and cow's milk allergy. The second part of the study involved 10 breast-fed infants who had atopic eczema and cow's milk allergy. In this group Lactobacillus GG was given to nursing mothers. METHODS: The severity of atopic eczema was assessed by clinical scoring. The concentrations of fecal alpha 1- antitrypsin, tumor necrosis factor-alpha, and eosinophil cationic protein were determined as markers of intestinal inflammation before and after dietary intervention. RESULTS: The clinical score of atopic dermatitis improved significantly during the 1-month study period in infants treated with the extensively hydrolyzed whey formula fortified with Lactobacillus GG. The concentration of alpha 1-antitrypsin decreased significantly in this group ($p = 0.03$) but not in the group receiving the whey formula without Lactobacillus GG ($p = 0.68$). In parallel, the median (lower quartile to upper quartile) concentration of fecal tumor necrosis factor-alpha decreased significantly in this group, from 709 pg/gm (91 to 1131 pg/gm) to 34 pg/gm (19 to 103 pg/gm) ($p = 0.003$), but not in those receiving the extensively hydrolyzed whey formula only ($p = 0.38$). The concentration of fecal eosinophil cationic protein remained unaltered during therapy. CONCLUSION: These results suggest that probiotic bacteria may promote endogenous barrier mechanisms in patients with atopic dermatitis and food allergy, and by alleviating intestinal inflammation, may act as a useful tool in the treatment of food allergy.