I. Basic Immunology

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.