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.