Editorial

Medical immunology: a new journal for a new subspecialty
Kendall A Smith

Address: The Division of Immunology, Department of Medicine Weill Medical, College of Cornell University, 1300 York Avenue, Box 41, New York, NY 10021, USA

E-mail: kasmith@med.cornell.edu

This new journal, *Medical Immunology*, was conceived to fill the large divide between the science of basic immunology and the application of this scientific knowledge to the diagnosis, treatment and prevention of diseases of the immune system. Why is there a gap between the basic science of immunology and the application of this science to medicine? The answers to this question are historical in large part, in that the immune system has only become recognized and defined within the last century. Moreover, the rate of accumulation of new information has accelerated markedly, with most of our present understanding acquired only within the past 20 years. Consequently, most of what we know about the immune system has only come into being within the professional lifetimes of the practicing medical community. Because of this recent explosion in knowledge, there simply has not been enough time for the translation to clinical medicine to occur.

There is a large community of basic scientists who have been educated in the last few decades and who have contributed to the current state of knowledge in immunology. By comparison, because of "turf" concerns amongst faculties of medical schools, immunology course time in medical schools has been miniscule if covered in the curriculum at all. Consequently, most practitioners have not received much formal education in immunology. My medical colleagues often tell me that the immunological acronyms and jargon are huge and obfuscating, and they have no idea what the immunologists are talking about.

There is definitely a gap between the immunologists and the clinicians.

Even so, because of the rapid advances that have been made, especially within the last 20 years, we now have a new understanding of how the immune system functions, and we have available to us many new molecules to use as therapeutics. As well, because of advances in molecular genetics, we now have the capacity to engineer new molecules and vaccines at will. Consequently, there is now a tremendous need for clinical research to determine how best to use these new therapeutics, particularly, without toxicity.

Because the modern science of immunology has only sprouted within the last 2–3 decades, most of those who contributed to our present view of the immune system are alive and well and still contributing. Therefore, by taking an historical view on this newest "organ system", it is easiest to gain an appreciation of what we now know, by focusing on those who have made a significant impact on medical immunology. As well, such a review should serve to illuminate those areas that promise to be the most fruitful areas for future development.

The discovery of the immune system and the dashed hopes of our medical immunologist forefathers
Immunology is the study of the physiology of the immune system, while the pathophysiology of the immune system is not of concern to many immunologists. Claude

Bernard made the connection between the physiology and pathophysiology of organ systems in his book, "An Introduction to Experimental Medicine", which was first published in 1865 [1]. At that time, the immune system had not yet been discovered, while the circulatory system, for example, had been scrutinized for 300 years. Sir Edward Jenner had opened the way to the immune system in 1798 with his description of cowpox virus vaccination against smallpox [2]. However, for most of the 19th century this phenomenon was considered a special case, and not applicable to other diseases. Microbes had yet to be understood as the causes of contagious diseases.

Louis Pasteur forwarded the germ theory in 1857 to counter the popular concept of "spontaneous generation" as responsible for fermentation. However 20 years passed before Robert Koch first proved that microbes could actually be responsible for causing disease in his seminal studies on anthrax [3]. This demonstration set the stage for Pasteur to introduce and promote the idea that it might be possible to attenuate microbes, which could then be used to prevent microbial infections by vaccination [4–6]. At about the same time, Eli Metchnikoff discovered phagocytosis, thereby introducing the concept that the host has cellular defenses against invasion by microbes [7]. Then, in 1890 with the discovery by Behring and Kitasato of antibody activity against diphtheria and tetanus toxins [8], serotherapy was soon initiated for these and other dread bacterial infections and found to be quite effective. Accordingly, 100 years ago at the beginning of the 20th century it was hoped that it would not only be possible to prevent infections by vaccination, but to also treat them effectively with antibodies. Medical immunology was born.

However, the attenuation of microbes proved elusive, as each microbe was found to be complicated and distinct. As well, Charles Richet and Paul Portier soon discovered anaphylaxis [9]. Thus, there appeared to be a downside to the repetitive injection of foreign molecules and microbes, and prophylaxis by vaccination was not so easily attained. Moreover, even though serotherapy was effective and many of those treated recovered from dread diseases such as diphtheria and scarlet fever, it was soon discovered by Arthus that the horse sera used for the serotherapies could produce an inflammatory syndrome itself [10], which was dubbed "*Serum Sickness*" by Clemmons von Pirquet and Bela Shick [11]. Also, at the same time, William Coley introduced the injection of bacterial extracts (Coley's toxins) as therapy for cancer [12]. Even though curative in some cases, Coley's toxins were never widely accepted because of the severity of the reactions induced.

Accordingly, it was realized that a good deal more knowledge was going to be necessary before the power of the immune system could be harnessed, and the hopes of physicians regarding the promise of manipulating the immune system for the prevention and treatment of disease faded.

The first half of the 20th century: understanding antibodies and bacteria

With the demise of immunotherapy the science of immunology was born at the beginning of the 20th century. For the next 50 years those interested in the immune system focused on trying to understand the physiology of the system. During this time, the focus was almost entirely on understanding the function and structure of antibodies, and the cellular immunology of Metchnikoff was forgotten. Karl Landsteiner's work stands out among the many contributors to our knowledge of serology, because he showed that antibodies are both tremendously diverse, capable of binding to almost all environmental molecules, and also exquisitely specific [13].

Simultaneously, in the first half of the 20th century the science of bacteriology was born and basic scientists commenced with the painstaking laboratory research characterizing these diverse microbes. By the 1950s, most bacteria had been identified, categorized, and characterized thoroughly. Then, with the advent of antibiotics, many bacterial diseases could finally be treated effectively. Also, antibiotics made extended cell cultures feasible for the first time, which were instrumental for both the discovery and propagation of viruses, so necessary for the creation of attenuated live viral vaccines. Only then, was Pasteur's dream of vaccines finally realized, and then only for viral infections. Moreover, the first successful vaccines for poliovirus and the other childhood exanthems were developed empirically, without knowledge as to how the immune system actually works. Consequently, immunology as a basic science had to develop before the application of this new knowledge to immunological diseases could occur.

Identifying the cells of the immune system

The advances in cell culture methods also led to the cultivation of lymphocytes, and the discovery by Peter Nowell in 1960 that lymphocytes were not end-stage differentiated cells as had been thought, but rather they were capable of rapid and extensive proliferation in response to mitogens and antigens [14]. As well, plasma cells, which by then were known to the source of antibodies [15], actually were shown to be derived from lymphocytes. Soon thereafter, Neils Jerne and Al Nordin developed an assay that could detect individual spleen cells as antibody producers [16], which essentially proved Sir McFarland Burnett's clonal selection hypothesis of antibody formation [17].

Then in 1970, B-lymphocytes (B cells) were identified by Raff and Pernis as precursors to plasma cells and characterized for the first time as cells with immunoglobulin (Ig) on their surface [18,19]. Soon thereafter T-lymphocytes (T cells) were identified for the first time by Raff and Pierre Vassali's group as lymphocytes lacking surface Ig, but having cell surface molecules distinct from B cells [20,21]. Within a few years, dendritic cells (DCs) were described by Steinman and Cohn as cells morphologically distinct from Metchnikoff's macrophages and microphages [22]. Natural Killer (NK) cells, which could kill target cells without activation by antigen, were discovered by Herberman and Keissling, Klein and Wigzell to be a distinct type of lymphocyte [23,24]. Thus, by the mid 1970s the major cells responsible for the immune response had been identified.

Histocompatibility restriction of the immune response
In 1959, Benacerraf and Gell showed that cell-mediated immune reactivity could be detected by using denatured proteins, while antibody reactivity could not [25,26]. Then, pioneering experiments by Benacerraf's group in the guinea pig in the early 1960s revealed a genetic component to the antibody response to synthetic polypeptide antigens [27,28]. Subsequently, McDevitt's group confirmed and extended these early findings to mice and showed that the immune responsiveness mapped to the major histocompatibility complex (MHC) gene loci [29,30].

Then, in the early 1970s histocompatibility between T cells and B cells was first found to be necessary for the antigen activation of antibody production by Katz, Hamaoka and Benacerraff [31], as well as between T cells and macrophages for the activation of antigen-specific T cell proliferation by Rosenthal and Shevach [32], and between virus-infected target cells and cytolytic T cells (CTL) for the cytolytic reaction by Zinkernagel and Doherty [33]. At the time, the molecular basis for this "histocompatibility restriction" of antigen recognition was not obvious, but the idea that antigens somehow could only activate T cells in the context of autologous molecules encoded by the MHC entered immunology for the first time.

Cloned antibodies and cloned antibody genes
There followed in 1975 the creation of plasmacytoma/B-cell hybrids (hybridomas), which produced monoclonal antibodies by Kohler and Milstein [34]. With the advent of monoclonal antibodies, the hope of "serotherapy" was rejuvenated. Thus, scientists as well as the business world had dreams of "magic bullets" that would target many intractable diseases, such as cancer. However, hybridomas could not be generated using human cells and the Human Anti-Mouse Antibody (HAMA) response that was induced

with the mouse antibody treatment was reminiscent of "Serum Sickness" instead.

In 1976, Hozumi and Tonegawa reported the amazing findings that revealed how the tremendous diversity of antibody molecules is generated: genes were found to rearrange and recombine [35]. This startling discovery set in motion a flurry of research that culminated in cloning of cDNA encoding immunoglobulin chains and ultimately the cloning of immunoglobulin genes by Tonegawa's group and Phil Leder's group [36–38]. Accordingly, almost a century of science was necessary to finally solve the mystery of the tremendous diversity but exquisite specificity of antibody molecules.

The advances contributed by many investigators in the 25 years since the initial identification of the immunoglobulin genes, have culminated in the ability to "humanize" mouse monoclonal antibodies by replacing most of the mouse gene sequences with human gene sequences. This manipulation considerably reduces the incidence of HAMA responses when the antibodies are used therapeutically. In addition, because of advances in molecular genetics, it is now possible to replace mouse immunoglobulin genes with human genes in embryonic stem cells, so that mice that produce human antibodies can be made [39,40]. Since the immune systems of these mice recognize as foreign everything that is "non-mouse", it is now possible to generate human monoclonal antibodies reactive with any and all human molecules and cells. The therapeutic potential of these antibodies is enormous, so that the dreams of the medical immunologists of a century ago may now finally be realized.

The flow cytometer and CDs
The combination of fluorochrome-conjugated monoclonal antibodies with the timely invention of the flow cytometer/Fluorescence-activated cell sorter (FACS) by Len Herzenberg and his colleagues revolutionized immunology [41]. For the first time, large numbers of individual cells within a population of mononuclear cells from blood, or spleen and lymph nodes could be identified and separated. Stuart Schlossman together with Ellis Reinherz and Jerry Ritz and their colleagues reported that monoclonal antibodies could be used to identify functional subsets of lymphocytes and leukemia cells [42–44], and they promoted cooperative efforts that led many investigators over the course of the next decade to identify hundreds of monoclonal antibodies that react with cell surface molecules or Clusters of Determinants (CD) that detect and discriminate separate lymphocyte subsets and functions. It is now routine to send blood to the clinical lab to determine the differential count of CD3+ T cells (both CD4+ and CD8+ subsets) [44,45], B cells (CD20+) [46], NK cells (CD16/56+) [47] and monocytes (CD14+)

[48], which is performed using the flow cytometer and some of the first lymphocyte subset-specific monoclonal antibodies reported.

Cloned T cells, cloned T cell antigen receptors (TCR) and cloned TCR Genes

In 1979 we reported the creation of the first monoclonal T cells [49]. Using lymphocyte conditioned media that Morgan and coworkers had found could promote long-term T cell growth [50], we had already generated the first antigen-specific cytolytic T lymphocyte lines (CTLL) by immunizing mice with allogeneic tumor cells [51]. Subsequently, upon limiting dilution cloning, we could show unequivocally that monoclonal T cells were reactive either with tumor-specific antigens or alloantigens [49]. At the time we speculated that monoclonal antigen-specific CTL might be used as therapy [52]. The generation of T cell clones that retained antigen-specific function reduced to the progeny of a single cell the tremendous diversity of T cell antigen recognition encountered when studying cell populations isolated from the blood or spleen. Consequently, this breakthrough quickly led to the unequivocal demonstration of MHC restriction of antigen recognition by Schwartz and coworkers [53].

Moreover, monoclonal antibodies reactive with T cell clones led to the discovery of the elusive T cell antigen receptor (TCR). In 1982, Allison and his co-workers reported the generation of monoclonal antibodies reactive with a T lymphoma cell line [54]. They speculated that the antibodies recognized a tumor-specific antigen, or perhaps the TCR. However, they had no way of testing the antibodies to prove their suspicions. Thus, the generation of "clonotypic" monoclonal antibodies reactive with IL2-dependent normal antigen-specific cytolytic T cell clones enabled Reinherz and co-workers in 1983 to identify the first human TCR [55,56]: their clonotypic monoclonal antibodies blocked antigen-specific activation of the cloned cytolytic T cells.

As well, Kappler and Marrack and their co-workers identified the murine TCR by generating clonotypic monoclonal antibodies reactive with an antigen-specific T cell hybridoma. These clonotypic antibodies were agonistic, and stimulated IL2 production, a TCR-specific functional assay [57]. Then, all three groups concurred simultaneously on their very similar findings, which indicated that there were both variable and constant portions to the TCR molecules they had identified on T lymphoma cells, T cell hybridomas and normal T cell clones [58–60].

Subsequently, in 1984 Hedrick and Davis applied classical nucleic acid hybridization kinetics and subtractive hybridization to search for the TCR genes, and cloned the cDNA encoding the first of the TCR chains, which re-

vealed that the TCR genes and molecules belong to the immunoglobulin super family [61].

Cloned NK cells

Soon after we had reported the first T cell clones, Gunther Dennert also used lymphocyte conditioned media to derive the first murine NK cell lines and clones, which allowed an unequivocal distinction to be made between these unique lymphocytes vs. T cells and B cells [62,63]. There followed the derivation of the first human NK cell lines and clones by Jerry Ritz and his colleagues [64,65], which enabled them to derive NK cell-specific monoclonal antibodies that could be used to distinguish these cells in a mixed population for the first time [65]. Subsequent studies with these NK cell clones revealed that NK cells produce a restricted set of cytokines that activate macrophages and DCs (see below). Therefore, innate immune reactivity would eventually be understood to revolve around a synergistic interaction between NK cells, macrophages and DCs (see below).

The interleukins: the hormones of the immune system

Although it was possible to culture T cell and NK cell clones using lymphocyte conditioned media as a source of growth factor(s), the identity of the molecules in the conditioned media responsible for the growth promoting activity remained obscure. Therefore, we used monoclonal T cells to construct an assay for the T cell growth factor (TCGF) activity in mitogen-activated lymphocyte conditioned media [66], which enabled the identification and discrimination of the first hormones of the immune system, interleukin-1 (IL1) and interleukin-2 (IL2) [67]. IL1, a product of activated macrophages originally termed Lymphocyte activating factor (LAF) [68], was found to augment the production of TCGF by T cells, hence the numerical order of these first two interleukins [57].

Over the next two decades many additional cytokines were discovered and found to be responsible for the regulation of the maturation, proliferation, differentiation and survival of B cells, T cells, NK cells, macrophages and dendritic cells [69–74]. The numbering of interleukins now includes IL27 and promises to continue. Some of the most important of these interleukins include IL4, initially described by simultaneously by Vitteta and Krammer, and Howard and Paul and their colleagues, which serves as a growth and differentiation factor for B cells and promotes the differentiation of monocytes to DCs [69,70], IL7, discovered by Namen,s group, which is a primary growth and differentiation factor for immature T cell and B cell progenitors [74], IL10, first described by Moore and coworkers, which serves as an important differentiation factor for B cells and regulates both T cell and dendritic cell function [75,76], IL12, discovered by Gately and Trinchieri and

their coworkers, which is an important growth and differentiation factor for cytolytic T cells and NK cells, and promotes the production of IFN-γ by NK cells and T helper-1 cells [73,76], and IL15, first described by groups led by Waldmann and and Grabstein, and which promotes the maturation of NK cells and the survival of memory CTLs [77,78]. IFN-γ is an important differentiation factor for both T cells and B cells, as well as macrophages and dendritic cells [79]. In addition, Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) described by Metcalf's team [80,81] promotes the proliferation and differentiation of macrophages and dendritic cells (DCs) from bone marrow myeloid precursors and blood monocytes [82].

The generation of the first monoclonal antibodies reactive with IL2 allowed its purification to homogeneity in milligram quantities in a single step, which was a first in cytokine research [83]. As each cytokine was discovered, monoclonal antibodies reactive with them aided their purification as well as detection using ELISA [84], which improved the analysis of cytokine physiology immensely. However, the development of a flow cytometry assay by O'Gara's group [85] that allows detection of cytokines within single cells via cytokine-reactive fluorochrome-conjugated monoclonal antibodies provided a quantum jump in the detection of antigen-specific T cells. The key technical improvement proved to be the addition of inhibitors of secretion such as monensin or Brefeldin A, which allow the accumulation of the cytokines inside the cells. The power of the single cell analysis of flow cytometry, combined with the capacity to examine intracellular as well as extracellular molecules has thus extended the analytical capability of the flow cytometer enormously.

Beutler, Cerami and their co-workers showed that pro-inflammatory cytokines such as cachectin or tumor necrosis factor-alpha (TNF-α), IL1 and IL6 produced by both lymphocytes and macrophages are the "endogenous pyrogens" that had led to so much toxicity from Coley's toxins, and to septic shock from bacteremia [86–89]. Based upon these findings, Marc Feldmann and his colleagues painstakingly developed the concept that inhibition of TNF-α activity would suppress downstream IL1 and IL6 production and the chronic inflammation of rheumatoid arthritis [90,91]. Most investigators at the time felt that the pro-inflammatory cytokines were redundant, so that blocking only one of them would have little effect. However, subsequently, they showed that humanized monoclonal antibodies reactive with TNF-α are effective in suppressing the inflammation and destruction of rheumatoid arthritis (RA) [92], while others found them effective in the treatment of inflammatory bowel disease [93].

Studies of T cell clones and the cytokines they produced also led to the discovery of two distinct types of cytokine-producing "helper" T cell clones by Mosmann and Coffman and their co-workers [94], which regulate the differentiation of B cells (Th-2 cells) [95], as well as T cells (Th-1 cells) [96]. Subsequently, Swain's [97,98] and Paul's [99] groups showed that the differentiation of a Th cell to a Th-2 cell was dependent upon the cytokine milieu. Thus, IL4 was found to be an important trophic cytokine, promoting the differentiation of Th-2 cells, which secrete high amounts of IL4, IL5, IL6 and IL10, all cytokines that influence the differentiation of B cells to antibody producing plasma cells.

By comparison, O'Gara, Murphy and co-workers found that IFN-γ influences Th cells to further secrete IFN-γ, thereby favoring activation of macrophages and delayed-type hypersensitivity. Furthermore, IL12 produced by IFN-γ-stimulated macrophages and DCs promotes Th-1 differentiation to high IFN-γ production, so that there is a reciprocal relationship between Th-1 cells and macrophages and DCs, with each favoring the other [100,101]. Pat Bucy and coworkers have examined the expression of cytokine genes using *in situ* hybridization and have found evidence for heterogeneity, consistent with the interpretation that there is not coordinate regulation of cytokine genes, and that each cell expresses a restricted repertoire of cytokine genes in a quantal manner [102]. More recent experiments reported from Glimcher's, Rao's and Murphy's groups have revealed that this regulation of gene expression is dependent upon newly discovered transcription factors and response elements [103–105]. Thus, it appears that the differentiation of Th cells depends upon the types and concentrations of cytokines that the cells are exposed to, which activate specific transcription factors and make the regulatory sites accessible.

T cell clones were also instrumental in the analysis of cytolytic granules [106] and the isolation of perforin, one of the active lytic molecules responsible for CTL activity by Podack and coworkers [107]. Therefore, the complex effector mechanisms of T cells became to be understood at the molecular level for the first time. These advances have spurred the hope that antigen-specific cytolytic T cells might be used for specific cellular therapy of cancer and infectious diseases. Actually, Riddell working with Greenberg's team has shown that cultured monoclonal cytolytic T cells are effective in the treatment of infection by cytomegalovirus (CMV) [108], while O'Reilly's group have pioneered the use of T cell clones for the treatment of Epstein-Bar Virus-induced T cell lymphoma [109]. Moreover, recently Yee and Greenberg and their coworkers have demonstrated that cloned T cells specific for antigens expressed by normal melanocytes and malignant melanoma cells are active in killing both cell types in vivo [110], and

Rosenberg's group has recently confirmed these observations [111]. Now that it is possible to identify the T cell clones after transfer in vivo using the new assays to detect antigen-specific cells [112], this area will undoubtedly see a great deal of activity in the future, using T cell clones to treat both viral infections and tumors.

The identification of the interleukins and pro-inflammatory cytokines led to the isolation of cDNA and genomic DNA clones encoding each molecule, which enabled the production of large quantities of recombinant proteins, thereby greatly facilitating research into the physiology of cytokines. Taniguchi first cloned the cDNA encoding IL2 [113], thereby showing the way toward cloning all of the subsequent cytokine molecules. As a result, recombinant cytokines have now become available for use therapeutically. Since IL2 was the first recombinant cytokine available, it was the first to be used in the clinic. Steven Rosenberg and Michael Lotze and their team administered IL2 in high doses to cancer patients, which resulted in the signs and symptoms of septic shock or SIRS, reminiscent of Coley's toxins [114]. However, like Coley's patients, some of those treated with high dose IL2, ~5%, appear to have been cured [115]. We have developed a different approach based on the high affinity of the IL2 receptor (IL2R) (see below), pharmacokinetic studies, and experiments by Doreen Cantrell, which showed that the magnitude of the IL2 biological response is dependent upon the IL2 concentration, the IL2R density and the duration of the IL2/IL2R interaction [116]. We administer 100-fold lower doses of IL2 continuously by daily subcutaneous injections without systemic toxicity [117,118].

Cytokine receptors

The discovery of cytokines also led to the discovery of cytokine receptors. Over the course of the 1980s and 1990s, the molecules responsible for binding the cytokines and for signaling the cell were identified and the genes encoding these molecules were cloned. The IL2R was prototypic, and was found to have a very high affinity ($Kd = 10^{-11}$ M) of binding and that the IL2 concentrations that bind to the receptor are identical to those that promote the biological response [119]. Initially we used radiolabeled IL2 in classic hormone/receptor binding experiments to demonstrate the IL2R. This assay was then used to identify the three chains of the receptor responsible for formation of the high affinity IL2 binding site [120–123]. Subsequently, these same methods were used to discover each additional cytokine receptor, as the cytokines became known.

Waldmann's group championed the development of humanized monoclonal antibodies reactive with the α-chain of the IL2R for the suppression of allograft rejection [124], which have now been shown to be effective in suppressing rejection of both renal [125] and cardiac allografts [126].

Moreover, soluble TNF-α R-Ig chimeric molecules have also been found efficacious in RA [127]. Thus, blocking cytokine activities with either monoclonal antibodies or soluble cytokine receptors promises to be an active area in medical immunology.

All of the work in the discovery of cytokine receptor chains recently has resulted in the first successful gene therapy experiment. Soon after Sugamura's group discovered and cloned the γ-chain of the IL2R [128], Leonard's group mapped the gene encoding this chain to the X chromosome and showed that there were mutations in this gene in patients suffering from X-linked Severe Combined Immundeficiency (SCID) [129]. Subsequent work revealed that this IL2R chain is also a component of the receptors for IL4, IL7, IL9, IL13 and IL15, so that this chain has become known as the common γ chain (γ$_c$) [130]. Then, Alain Fischer's group successfully circumvented the genetic immunodeficiency using retrovirus transferred γ$_c$ cDNA into bone marrow stem cells [131].

Signaling the cells

The discovery of the antigen receptors and the cytokine receptors initiated a quest still ongoing to identify the intracellular signaling molecules and the transcription factors activated by the triggered receptors. In this search, many of the immunosuppressive drugs already known were found to block specific signaling molecules and transcription factors activated in lymphocytes and macrophages. For example, glucocorticoids have been the mainstay of immunosuppressive and anti-inflammatory therapies for the past 50 years. Our early experiments revealed that glucocorticoids inhibit the TCR activation of IL2 production [132]. Subsequent work by both Michael Karin's and Al Baldwin's groups showed that glucocorticoids block the TCR activation of Activating Protein-1 (AP-1) and the Nuclear Factor-kappa of B cells (NF-κB) [133–135]. Similarly, Hermann Wagner and his co-workers showed that the immunosuppressive antibiotic cyclosporin-A (CSA) is effective because it also blocks the TCR signaling of cytokine production [136], and subsequently, Gerald Crabtree's group found that CSA inhibits the TCR activation of the Nuclear Factor of Activated T cells (NF-AT) [137].

Subsequent work using gene-deleted mice by Gerondakis' and Hsiou-Chi Liou's groups have shown that c-Rel, which is the only member of the NF-κB transcription factor family restricted to the hematopoietic system, is obligatory for TCR and BCR signaling of cytokine gene expression, proliferation, differentiation and cellular survival [138–142]. Thus, NF-κB, originally described by Sen and Baltimore as involved in Ig gene expression [143], has proven to be one of the most important transcriptional activating factor families in the immune system and is oblig-

atory for optimal functioning of lymphocytes, macrophages and dendritic cells.

All of these data underscore the importance of targeting these signaling molecules and transcription factors for the development of new traditional small molecule drugs capable of either suppressing or enhancing immune responsiveness. Recently, the power of our new knowledge in designing effective immunosuppressive regimens was demonstrated in the combination of the drug FK506, which like CSA blocks NF-AT activation and thereby IL2 gene expression, with the monoclonal antibody reactive with the α-chain of the IL2R thereby competing for ligand activation of the IL2R, and Rapamycin, a drug that blocks IL2/IL2R signaling. This is the first immunosuppressive combination therapy that has avoided using glucocorticoids (which are diabetogenic), and it has been found effective in preventing rejection of pancreatic islet β-cell grafts as a new therapy for Type I diabetes [144].

Signaling via the interferon receptors and the interleukin receptors involve some pathways in common with the TCR and BCR, but a whole new series of molecules were discovered as critical for cytokine signaling that are not triggered via the antigen receptors. Experiments focused on signaling via interferons by Darnell revealed a family of molecules that upon triggering via interferon were found to translocate to the nucleus and regulate gene expression [145]. Subsequently, extensive work by Darnell's group with elegant genetic experiments by Stark and Kerr revealed that this family termed Signal Transducers and Activators of Transcription (STAT) is critical for signaling new gene expression by the cytokine receptor super-family [146–148].

The Janus kinases (JAK) were identified by Jim Ihle's group as important for signaling via erythropoietin (EPO) receptors [149], and subsequently many of the receptors for the interleukins and hematopoietic colony stimulating activities that are members of the same super-family of cytokines and receptors were found to activate distinct members of the JAK family [150]. These experiments thus showed how the JAKs connect the cytokine receptors with the STATs. As pointed out by O'Shea, future research into agents that either inhibit or enhance the activities of these molecules are obvious areas for drug development to identify new compounds useful as immunomodulators [151].

Antigen processing and presentation

Despite all of these advances, particularly in understanding that the TCR belongs to the same family as do antibody molecules, and that the hormones of the immune system regulate lymphocyte proliferation and differentiation, the requirement for MHC restriction of antigen rec-

ognition by T cells remained an enigma. However, there were several discoveries that gradually clarified the molecular mechanisms responsible for these phenomena. First, in the 1960s, Stuart Schlossman and coworkers had shown that very short peptides with as few as 6 amino acids, could be recognized during cell mediated immunity [152]. Also, as early as 1968, Unanue and Askonas [153] showed that despite rapid degradation of radiolabled antigen by macrophages, these cells remained immunogenic for as long as 3 weeks. Then, in 1982 Zeigler and Unanue [154] showed that the catabolism of antigen by macrophages was actually *necessary* for their capacity to present antigen to T cells.

Moreover, already in 1969, George Mackaness had shown that antigen-activated lymphocytes activate macrophages to kill intracellular microbes [155]. Soon thereafter, this macrophage activation was found to be due to a soluble lymphocyte-derived factor by John David's lab [156,157]. Then, Michael Tovey working with Ion Gresser's group showed that interferons, which had such potent antiviral activity, also promoted the expression of histocompatibility antigens [158,159]. In 1983 this macrophage activating factor (MAF) activity was shown to be interferon-γ (IFN-γ) by Henry Murray, Carl Nathan and Bob Schreiber their co-workers [79,160,161]. Now it is known that IFN-γ upregulates just about everything that macrophages are capable of doing, including antigen processing and presentation, in addition to phagocytosis and intracellular killing.

In 1985, Unanue's group demonstrated that "processed" protein antigens of short peptides (10 amino acids) actually bind to MHC molecules inside the cell, prior to appearing on the cell surface [162]. Subsequently, in 1987 Pam Bjorkman, working as a doctoral student with Don Wiley and Jack Strominger, solved the 3 dimensional crystal structure of the first MHC encoded molecule [163]. The visualization of the structure of a MHC molecule was a revelation: Two parallel alpha helices form a pocket where short antigenic peptides reside. Thus, T cells recognize peptide antigens bound to MHC-encoded molecules, and antigen presenting cells (APCs) process complex proteins by degradation to short peptides of 8–15 amino acids. This information, combined with structural information from Ig molecules and the knowledge that the TCR chains are homologous to Ig molecules provided a picture for the first time of the way in which the TCR docks onto the peptide-MHC molecular complex [164].

Lymphocyte development

With the generation of monoclonal antibodies recognizing distinct CDs, Reinherz and coworkers introduced the notion of using these markers to track the maturation of lymphocytes [165]. Scollay and Shortman then led the

way with detailed studies of murine thymocyte subsets distinguished by their differential expression of surface markers detected by flow cytometry [166,167]. However, the cause and effect of changes in surface marker expression vs. functional maturation of the TCR and accessory molecules was more difficult to assess. Ada Kruisbeek and coworkers reported seminal experiments, showing that monoclonal antibodies reactive with MHC Class II molecules administered to neonatal mice prevented the development of CD4+ T cells, thereby providing proof of the concept that signals passing from the thymic stromal MHC molecules to thymic TCRs was important for T cell maturation [168,169]. Subsequently, using mice expressing transgenic TCRs, von Boehmer's group showed unequivocally that the interaction of the TCR on the immature thymocyte with MHC molecules on thymic supporting cells determines the positive selection of thymocytes into "single-positive", CD4+ or CD8+ T cells recognizing non-self epitopes, and negative selection of self-reactive TCRs [170–172].

In the 25 years since the realization that antibody diversity is generated by the rearragement and recombination of immunoglobulin genes, a multitude of investigators have studied the processes by which these phenomena occur. Early B and T cell precursors generate antigen recognition diversity by assembling the exons that encode Ig or TCR variable regions from individual variable (V), diversity (D), and joining (J) gene segments through recombination-activating gene-1 (RAG-1) and RAG-2 proteins as shown by Baltimore's group [173].

Much of the work on lymphocyte development was performed initially without consideration of a role played by cytokines. However, with Namen's discovery of IL7 [74,174], first as a growth and differentiation factor directed toward immature B cells, then as a cytokine that also promotes the proliferation and survival of immature thymocytes, many of the developmental changes found to take place are now known to actually be mediated by IL7. Most of the severe combined immunodeficiencies (i.e. 70–80%) have been traced to mutations that affect the IL7 pathway. Consequently, within the past several years, Crystal Mackall and her group have reported on the effects of the administration of IL7 to both normal and T cell depleted mice [175,176]. The data indicate that IL7 may well be an effective cytokine to accelerate the recovery of the immune system after bone marrow transplantation or acquired immunodeficiency [177].

Dendritic cell lines
In 1992 Blanchereau's group working with human cells [178] and independently, Gerold Schuler, working with murine cells along with Kayo Inaba and Ralph Steinman [179], reported that GM-CSF promotes the proliferative expansion of mononuclear cells with the characteristics of dendritic cells (DCs). DCs had remained a curiosity for almost 20 years, because they are present in such low numbers in lymphoid cell populations, usually < 1%. Consequently, obtaining enough cells for molecular studies was difficult. By comparison, tissue macrophages and DCs are very prevalent, but difficult to access. However, the discovery that GM-CSF acts as a myeloid lineage-specific growth and differentiation factor like EPO is for erythroid cells, and the subsequent finding by Schuler and his coworkers that the addition of IL4 prevents differentiation to macrophages thereby facilitating the GM-CSF-driven default pathway of differentiation to DCs, fueled an explosion of work in this area [180].

Originally, it was thought that DCs were a separate cell lineage that differentiated from a common myeloid stem cell precursor, present in very small numbers in bone marrow and blood [179]. However, from studies by Lanzavecchia's group, it is now evident that blood monocytes differentiate into "immature DCs" when acted upon by GM-CSF and IL4 [181,182]. In the absence of IL4, GM-CSF promotes the differentiation of monocytes to macrophages. Thus, DCs are not a separate myeloid cell lineage, but rather a differentiation pathway of myeloid cells, much like the differentiation of Th-1 and Th-2 cells, which is also under the supervision of cytokines such as IL4 and IFN-γ.

Moreover, the switch from immature DCs which are efficient at antigen capture, to "mature DCs", which acquire attributes that facilitate antigen presentation, is signaled by a variety of stimuli that are associated with severe infection, including whole bacteria [183], bacterial endotoxin [182], bacterial DNA [184], and especially pro-inflammatory cytokines such as IL1 and TNF-α, while IL10 inhibits this differentiative process [185]. Of particular importance, mature DCs secrete large amounts of IL12, and express high levels of MHC Class II molecules, as well as CD40, co-stimulatory and adhesion molecules, all of which make for very efficient antigen presentation and activation of T cells. As well, it is important to emphasize that many of the ligands that impact mature DCs signal the activation of NF-κB transcription factors, of which c-Rel is the most important.

Recently, Fred Siegal's group reported a new type of DC, termed a plasmacytoid DC (pDC) that is thought to derive from a lymphoid lineage, in that it expresses CD4 and CD123, but not CD11c, a marker for the myeloid lineage [186]. pDCs are also distinguished from their myeloid cousins by their marked production of IFN-α upon induction with viruses and bacteria. By selecting for pDCs, the production of IFN-α is enhanced ~600-fold, while monocytes-derived DCs produce barely detectable amounts. As

well, pDCs are distinguished by their differentiation in the presence of IL3 and CD40L, while myeloid DCs require GM-CSF and IL4. Given the growing awareness of the phenotypic and functional diversity of DCs, the derivation of distinct DC clones seems warranted.

In retrospect, given the role of DCs in the activation of lymphocytes and their production of pro-inflammatory cytokines such as TNF-α, the toxicity of Coley's toxins (heat-killed bacterial extracts) can now be better understood. In addition, given the role of adjuvants that contain bacterial extracts, such as Complete Freund's Adjuvant (CFA), in promoting maximal activation of the immune system by vaccines, investigators are now exploring DCs as a new adjuvant form of cellular therapy [187]. Thus far, DCs have been employed in cancer therapy studies, with some promising initial results, but many issues remain to be explored, including optimal cell numbers, ease of preparation, frequency of administration, and potential side effects [188].

B cell clones and B cell Differentiation

With much of the immunologic community focused on T cells, B cells received comparatively much less attention in the '80s. However, in 1991, Jacques Banchereau's group reported that it is possible to promote the long-term growth and clonal isolation of normal human B cells by stimulating them with a combination of IL4 and monoclonal antibodies reactive with CD40, a member of the TNFR family [189,190]. Thus, large numbers of normal B cells could finally be obtained in a manner analogous to the long-term growth of T cells and NK cells. Soon thereafter, Seth Lederman working in Len Chess's group, reported the derivation of a monoclonal antibody reactive with a T helper cell surface activation molecule, which is now known as CD40 ligand (CD40-L) [191,192]. Thus, T cell "help" for B cells was shown to be mediated by both a soluble cytokine provided by the Th cells (IL4), and by a T cell surface ligand (CD40-L). Almost immediately, Raif Geha's group showed that the genetic defect of the "Hyper-IgM syndrome", where there is a defect in B cell Ig isotype switching, is ascribable to mutations in T cell expression of CD40-L [193].

After mature B cells leave the bone marrow and populate the peripheral lymphoid tissues, they further diversify the antibody repertoire through two antigen-dependent processes that usually occur in the germinal center of secondary lymphoid organs. These are Ig V(D)J gene somatic mutation, which increases the antibody affinity for antigen by introducing point mutations within the V(D)J exon [194], and Ig heavy chain class switching, which modifies the antibody effector functions by substituting the constant region of IgM with that of IgG, IgA, or IgE [195]. Hypermutated and class-switched germinal center

B cells give rise to long-lived memory B cells or terminally differentiate to plasma cells, which secrete large amounts of immunoglobulin.

Experiments using cloned human lymphoma cells by Paolo Casalli and his group have revealed that somatic hypermutation is signaled by activation of the BCR, but that help is required from T cells via engagement of the CD40L-CD40 and CD28-CD80 ligand receptor systems [196]. By comparison, this same group found that class switch recombination (CSR) is dependent upon the CD40L-CD40 triggering by Th cells, but as well, cytokines such as IL10, IL4, and TGF-β are required [197]. Now Andrea Cerutti with Casalli and their coworkers have found that IFN-α, IFN-γ or CD40L upregulate the TNF family members BlyS and APRIL on DCs. Then in the presence of IL10 or TGF-β, BlyS and APRIL on DCs induce CSR from Cμ to Cγ and/or Cα genes in B cells, whereas CSR to Cε requires IL4 [198]. Secretion of class switched antibodies requires additional stimulation by BCR engagement and IL15. Thus, unlike T cell-dependent class switching, T cell-independent class switching, which occurs in splenic marginal zone or intestinal lamina propria B cells, provides prompt protection against invading pathogens. Similar findings have also been reported by John Kearney and his group [199]. The implications for these new findings for host defenses, especially against encapsulated bacteria, are obvious.

Innate immunity: toll-like receptors (TLR)

In a separate, but related area of research, recent detailed genetic studies finally solved the mystery underlying the C3H-HeJ mouse, which is resistant to shock induced by bacterial endotoxin or lipopolysccharide (LPS) [200]. The story originated in 1976 with the first description of a soluble factor induced by the injection of LPS that induced the necrosis of tumors by Lloyd Old's group [201]. Subsequently, Tony Cerami's group reported that LPS induced the inhibition of lipoprotein lipase activity by a factor that they termed cachectin, which led to a paradoxical hyperlipemia in a situation of a chronic wasting syndrome [202].

Subsequently, the cDNA for tumor necrosis factor-alpha (TNF-α) was cloned and sequenced by David Goedell's group [203]. Soon thereafter, Beutler purified, cloned and sequenced cachectin [204], and it was realized that TNF-α and cachectin are one in the same [87]. More than a decade later, after painstaking chromosome walking experiments Bruce Beutler's group solved the mysterious lack of the ability of C3H-HEJ mice to respond to LPS only in 1998 [200]. The genetic defect resides in a receptor originally discovered in fruit flies, the Toll receptor family. Thus, the LPS receptor is now known to be the Toll-Like Receptor-4 (TLR-4). This receptor is expressed at high levels on APCs, especially mature DCs. Moreover, TLRs acti-

vate the NF-κB family of transcription factors in APCs, particularly c-Rel.

This discovery has led to a virtual cornucopia of research. For example, Herman Wagner and his collaborators have shown that there are TLRs for bacterial DNA, which has a preponderance of CpG sequences (TLR-9) [205], and Flavell's group has found that double-stranded RNA, long known to induce interferon and augment immune responses, binds to TLR-3 [206]. These receptors recognize repeating molecules, and they are now known as pattern recognition receptors. Doubtless, activation of TLRs will be a fruitful future area of research for those interested in both augmenting and suppressing immune responses.

Structure-activity relationships of the peptide-MHC-TCR complex

During this same interval, the structure-activity-relationships between the TCR on CD8+ T cells interacting with antigenic peptides bound to Class I MHC-encoded molecules and CD4+ T cells interacting with antigenic peptides bound to Class II MHC-encoded molecules were elucidated for the first time by groups led by Don Wiley, Ian Wilson and Ellis Reinherz [207–209]. Analysis of the intermolecular interaction at the atomic level is revealing subtleties that promise to explain such fundamental immunological issues as positive and negative selection during T cell maturation in the thymus, the capacity of CD4+ T cell TCRs to recognize multiple peptide-MHC (p-MHC) ligands, and how alloreactivity differs from the recognition of foreign peptides bound to self MHC [210].

In addition, John Altman, working in Mark Davis' group capitalized on the new understanding of how p-MHC complexes bind to the TCR to develop a new way to identify antigen-specific T cells [211]. By forming tetramers of MHC molecules, which can then be loaded with antigenic peptides, it is possible to improve the affinity of p-MHC-TCR binding so that the tetramer-bound cells can be detected by flow cytometry. This assay can then be used to enumerate the frequency of antigen-reactive T cells before, during and after an immune reaction. As well, Florian Kern and coworkers improved the detection of antigen activated T cells by using mixtures of overlapping peptides to stimulate cytokine production by both CD4+ and CD8+ T cells, which can then be detected by using ELISPOT and flow cytometry [212–214]. Accordingly, just as in the determination of the amount of antibody produced after antigenic exposure, it is now possible for the first time to quantify the T cell immune response.

Recent experiments by Mark Davis, Michael Dustin, Paul Allen, and their co-workers focused on the question of the threshold of TCR activation by p-MHC complexes has revealed that a TCR "synapse" forms, creating a specialized junction between a T cell and an antigen-presenting cell [215]. Immunological synapse formation is a dynamic mechanism that allows T cells to distinguish activating ligands from those that are not of sufficient "strength" to result in immunological recognition. Fully formed, the T cell synapse consists of a central cluster of TCR-p-MHC molecules surrounded by a ring of adhesion molecules. However, prior to engagement, TCR-p-MHC complexes form an outer ring of the nascent synapse. Transport of these complexes into the center of a cluster, so that the adhesion molecules form an outermost stabilizing ring is dependent upon TCR-p-MHC reaction kinetics and is at the essence of determining "immune recognition".

Fc receptors (FcR): connecting humoral and cellular immunity

From the humoral side of immunity, it was thought since the days of Metchnikov, Arthus, Shick and von Pirquet that the inflammatory responses observed during allergic and autoimmune reactions were due to antigen-antibody (Ag-Ab) complexes, which activated polymorphonuclear leukocytes (PMNs, the microphages of Metchnikov) via complement (C') ("Arthus Reaction"). In a seminal series of reports by Jeff Ravetch and his group, the pathogenesis of these inflammatory phenomena are now known to be due to the activation of macrophages and mast cells via antigen-IgG complexes binding to and triggering Fc receptors (FcR), which then results in their production of proinflammatory cytokines [216–218]. Therefore, there is a synergism between the humoral and cellular arms of the immune response that mediates the end result, which is the release of inflammatory cytokines. By comparison, their work has shown that C' works together with "natural" IgM antibodies to lyse bacteria and other cellular targets without the participation of FcR on macrophages or PMNs [219].

In addition, both IgG and IgE FcRs participate in the activation of mast cells, leading to their degranulation and release of the vasoactive amines that produce the immediate hypersensitivity reaction so characteristic of allergic reactions [220]. Finally, as a result of Ravetch's work, there are now known to be *inhibitory* FcRs that bind Ag-IgG complexes that serve to attenuate humoral immunity [221], so that manipulations that activate either the stimulatory and inhibitory FcRs are prime targets for the discovery of new immunomodulatory agents.

Because the common pathway of all of these phenomena initiated by the formation of Ag-Ab complexes is mediated via pro-inflammatory cytokines, it is not too much of a stretch to imagine that monoclonal antibodies reactive with TNF-α or soluble TNFRs may be effective in the treatment of such diseases as polyarteritis nodosa, even before we understand the underlying pathogenesis, just as they

are for RA and IBD, two other idiopathic inflammatory conditions. As well, drugs such as Cyclosporine A and FK506 that block signaling via the FcRs should be effective in the treatment of allergic and other inflammatory diseases.

The genomic age

Having identified the cells responsible for mediating the host defenses, the cytokines that direct their maturation, proliferation, differentiation and survival, the cell surface receptors responsible for transferring extracellular signals to the cell interior, and the signaling molecules/transcription factors that serve as second messengers emanating from the receptors, the identification of the genes that encode the molecules activated now represents a new area of research that promises to have major impact on the evolution of medical immunology.

Following the lead of Chuck Stiles who 20 years ago first cloned genes expressed by fibroblasts stimulated with Platelet-Derived Growth Factor [222], Carol Beadling initiated experiments a decade ago in our lab to identify and clone IL2-induced genes [223]. At the same time, Jacques Theze's group have also pioneered this approach [224]. Now, it is evident that DNA arrays will provide the necessary technology to identify the genes responsible for all of the complex processes that we recognize as the immune response. Thus, we should anticipate the discovery of many new genes and gene products that can serve as targets for future therapeutic intervention into the immune system.

Advances in molecular genetics have already made it possible to express or delete specific genes in mice so that reductionism can now be combined with reconstructionist science to provide insight into disease mechanisms. For example, Stefan Ehler's group has performed a tour de force in experiments directed at understanding the mechanisms responsible for "Koch's phenomenon". Using mice with deletions of individual genes thought to be involved, they demonstrated unequivocally that T cells and IFN-γ are obligatory for the caseous necrosis of tuberculoid granuloma, long an unexplained aspect of a classic cell-mediated immune reaction involving the interaction between T cells and macrophages in TB [225]. These experiments show the way to the determination of the cells and molecules that play a role in other immunological disease processes, so that rational interventions can be devised.

A century after Pasteur proposed that it might be possible to attenuate microbes, which could then be used as vaccines, because of genetic engineering, it is now possible to remove "virulence genes" from microbes and replace them with genes that can be expressed in their place, thereby immunizing with "safe" microbes. For example, one of the viruses that is being developed as a vaccine for the Human Immunodeficiency Virus (HIV) is a modified version of Jenner's cowpox virus, vaccinia [226]. Another, similar virus being developed is the canarypox virus [226]. Since the poxviruses are large, with > 200 genes, they make it possible to remove and replace genes without markedly altering the infectivity of the virus or the capacity of the altered virus to express the newly inserted genes [227–229]. Accordingly, Pasteur's dream of vaccines against the entire microbial world may finally become a reality.

An additional area where the genomic revolution promises to have a major impact is on the identification of antigenic epitopes, especially for T cells. As the genomic sequences of all of the various microbes become complete, it will be possible to use the sequence information to analyze potential peptide sequences for possible binding to the various MHC molecules, as well as whether there are side chains capable of impacting TCRs. Thus, the idea of "molecular mimicry" as a cause of autoimmunity will finally become testable. As well, many allergies due to cross-reactive epitopes of distinct allergens will finally become illuminated.

Conclusions

From this historical perspective, one can now see how the vision of our forefathers was not misplaced. Now, it *is* possible to generate and use antibodies as therapy, and it *is* possible to attenuate microbes to construct vaccines for the prevention of infectious diseases. However, in addition to these approaches envisioned 100 years ago, now that we have applied a reductionist approach to such a complex biological system over the past 30 years, we have identified the cells that comprise the immune system, and we have isolated individual clones of all of the major cell types that participate. As well, we have reconstituted the immune response *in vitro* and we have been able to determine how the cells recognize foreign molecules, and how they communicate with one another through cell surface ligand/receptor systems and through secreted hormones. Moreover, we have discerned how the system responds to the introduction of foreign material, first by massively expanding the number of cells reactive to the invading molecules, and then by their differentiation into cells that produce an amazing array of immunologic effector molecules. Consequently, we now have available to us a much more enlightened understanding of the physiology of the immune system, and as well, multiple cells and molecules to craft new approaches to the diagnosis, therapy and prevention of diseases that impact the immune system. It is now timely for a renaissance of Medical Immunology. Our new journal is meant to provide for a forum and a medium to voice this new subspecialty of medicine that

will be freely available to the lay public, scientists and clinicians alike.

References

1. Bernard C: *An Introduction to the Study of Experimental Medicine. New York: Dover Publishers Inc.* 1865
2. Jenner E: *An Inquiry into the Causes and Effects of Variolae Vaccinae, a Disease Discovered in Some Western Counties of England. London: Sampson Low* 1798
3. Koch R: **Die aetiologie der milzbrand-krankheit, begrundet auf die entwicklungsgeschichte des bacillus antracis.** *Beitrage zur Biologie der Pflanzen* 1877, **2:**277-310
4. Pasteur L, Joubert , Chamberland : **La theorie des germes et ses applications a la medicine et a la chirurgie.** *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences* 1878, **86:**1037-1043
5. Pasteur L: **Sur les maladies virulentes, et en particulier sur la maladie appelee vulgairement cholera des poules.** *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences* 1880, **90:**249-248
6. Pasteur L, Chamberland , Roux : **De l'attenuation des virus et de leur retore a la virulence.** *Comptes Rendus des Seances de L'Academie des Sciences* 1881, **92:**430-435
7. Metchnikoff E: *Immunity in Infective Diseases. Cambridge, UK: Cambridge University Press* 1905
8. Behring EAS, Kitasato K: **Ueber das zustandekommen der diphtherie-immunaitat und der tetanus-immunitat bei thieren.** *Deutsche Medizinische Wochenschrift* 1890, **16:**113-114
9. Portier P, Richet C: **De l'action anaphylactique de certains venins.** *Comptes Rundus des Seances de la Societe de Biologie et des Filials* 1902, **54:**170-172
10. Arthus M: **Injections repitees de serum de cheval chez le lapin.** *Comtes Rendus des Seances de la Societie de Biologie et de ses Filiales* 1903, 817-820
11. von Pirquet C, Shick B: *Serum Sickness. Baltimore: The Williams and Wilkins Co.* 1951
12. Coley W: **The treatment of malignant tumors by repeated inoculations of erysipalas: with a report of ten original cases.** *Am J Med Sci* 1893, **10:**487-511
13. Landsteiner K, van der Scher J: **On cross reactions of immune sera to azoproteins.** *J Exp Med* 1936, **63:**325-339
14. Nowell PC: **Phytohemagglutinin: An initiator of mitosis in cultures of normal human leukocytes.** *Cancer Research* 1960, **20:**462-468
15. Fagraeus A: **The plasma cellular reaction and its relation to the formation of antibodies in vitro.** *J Exp Med* 1948, 1-13
16. Jerne NK, Nordin AA: **Antibody formation in agar by single antibody-producing cells.** *Science* 1963, **140:**405
17. Burnet FM: **A modification of Jerne's theory of antibody production using the concept of clonal selection.** *Aust J Sci* 1955, **20:**67-77
18. Raff M, Sternberg M, Taylor RB: **Immunoglobulin determinants on the surface of mouse lymphoid cells.** *Nature* 1970, **225:**553-555
19. Pernis B, Forni L, Amante L: **Immunoglobulin spots on the surface of rabbit lymphocytes.** *J Exp Med* 1970, **132:**1001-1018
20. Raff M: **Surface antigenic markers for distinguishing T and B lymphocytes.** *Transplant Rev* 1971, **6:**52-59
21. Lamelin J, Lisowska-Berntstein B, Matter A, Ryser J, Vassalli P: **Mouse thymus-independent and thymus-derived lymphoid cells. I. Immunofluorescent and functional studies.** *J Exp Med* 1972, **136:**984-1007
22. Steinman R, Cohn Z: **Identification of a novel cell type in peripheral lymphoid organs of mice.** *J Exp Med* 1973, **137:**1142-1162
23. Herberman RB, Nunn ME, Holden HT, Lavrin DH: **Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells.** *Int J Cancer* 1975, **16:**230-239
24. Kiessling R, Klein E, Wigzell H: **Natural killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype.** *Eur J Immunol* 1975, **5:**112-116
25. Benacerraf B, Gell P: **Studies on hypersensitivity. I. Delayed and Arthus-type skin reactivity to protein conjugates in guinea pigs.** *Immunol* 1959, **2:**53-63
26. Gell P, Benacerraf B: **Studies on hypersensitivity. II. Delayed hypersensitivity to denatured proteins in guinea pigs.** *Immunol* 1959, **2:**64-70
27. Kantor FS, Ojeda A, Benacerraf B: **Studies on artificial antigens I. Antigenicity of DNP-polylysine and DNP copolymer of lysine and glutamic acid in guinea pigs.** *J Exp Med* 1963, **117:**55-64
28. Green I, Paul WE, Benacerraf B: **The behavior of hapten-poly-L-lysine conjugates as complete antigens in genetic responder and as haptens in non-responder guinea pigs.** *J Exp Med* 1966, **123:**859-879
29. McDevitt HO, Tyan ML: **Genetic control of the antibody response in inbred mice: Transfer of response by spleen cells and linkage to the major histocompatability (H2) locus.** *J Exp Med* 1968, **128:**1-11
30. McDevitt HO, Chinitz A: **Genetic control of the antibody response: Relationship between immune response and histocompatability (H-2) type.** *Science* 1969, **163:**273-279
31. Katz D, Hamaoka T, Benacerraf B: **Cell interactions between histoincompatible T and B lymphocytes II. Failure of physiologic cooperative interactions between T and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates.** *J Exp Med* 1973, **137:**1405-1418
32. Rosenthal A, Shevach E: **Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes.** *J Exp Med* 1973, **138:**1194-1212
33. Zinkernagel R, Doherty P: **Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system.** *Nature* 1974, **248:**701-702
34. Kohler G, Milstein C: **Continuous culture of fused cells secreting antibody of predefined specificity.** *Nature* 1975, **256:**495-499
35. Hozumi N, Tonegawa S: **Evidence for somatic rearrangement of immunoglobulin genes coding for variable and constant regions.** *Proc Natl Acad Sci U S A* 1976, **73:**3628-3632
36. Tonegawa S, Brack C, Hozumi N, Schuller R: **Cloning of an immunoglobulin variable region gene from mouse embryo.** *Proc Natl Acad Sci U S A* 1977, **74:**3518-3523
37. Bernard O, Hozumi N, Tonegawa S: **Sequences of mouse immunoglobulin light cain genes before and after somatic changes.** *Cell* 1978, **15:**1133-1139
38. Seidman J, Edgell M, Leder P: **Immunoglobulin light chain structural gene sequences cloned in a bacterial plasmid.** *Nature* 1978, **271:**582-586
39. Jakobovits A, Moore AL, Green LL, Vergara GJ, Maynard-Currie CE, Austin HA, Klapholz S: **Germ-line transmission of a human-derived yeast artificial chromosome.** *Nature* 1993, **362:**255-258
40. Green LL, hardy MC, Maynard-Currie CE, Tsuda H, Louie DM, Mendez MJ, Abderrahim H, Noguchi M, Smith DH, Zeng NE, David NE, Sasai H, Garza D, Brenner DG, Hales JF, McGuinness RP, Capon DJ, Klapholz S, Jakobovits A: **Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACS.** *Nature Genetics* 1994, **7:**13-21
41. Lanier L, Engleman E, Gatenby P, Babcock G, Warner N, Herzenberg L: **Correlation of functional properties of human lymphoid cell subsets and surface marker phenotypes using multiparameter analysis and flow cytometry.** *Immunol Rev* 1983, **74:**143-160
42. Schlossman S: **Characterization and function of human lymphocyte subsets.** *Prog Clin Biol Res* 1981, **58:**3-6
43. Nadler L, Ritz J, Griffin J, Todd R, Reinherz E, Schlossman S: **Diagnosis and treatment of human leukemias and lymphomas utilizing monoclonal antibodies.** *Prog Hematol* 1981, **12:**187-225
44. Reinherz EL, Hussey RE, Schlossman SF: **A monoclonal antibody blocking human T cell function.** *Eur J Immunol* 1980, **10:**758-762
45. Reinherz EL, Kung PC, Goldstein G, Schlossman SF: **Separation of functional subsets of human T cells by a monoclonal antibody.** *Proc Natl Acad Sci USA* 1979, **76:**4061-4065
46. Kung P, Goldstein G, Reinherz EL, Schlossman SF: **Monoclonal antibodies defining distinctive human T cell surface antigens.** *Science* 1979, **206:**347-349
47. Ritz J, Schmidt R, Michon J, Hercend T, Schlossman S: **Characterization of functional surface structures on human natural killer cells.** *Adv Immunol* 1988, **42:**181-211

48. Breard J, Reinherz E, Kung P, Goldstein G, Schlossman S: **A monoclonal antibody reactive with human peripheral blood monocytes.** *J Immunol* 1980, **124**:1943-1948

49. Baker PE, Gillis S, Smith KA: **Monoclonal cytolytic T-cell lines.** *J Exp Med* 1979, **149**:273-278

50. Morgan DA, Ruscetti FW, Gallo R: **Selective in vitro growth of T lymphocytes from normal human bone marrows.** *Science* 1976, **193**:1007-1008

51. Gillis S, Smith KA: **Long term culture of tumour-specific cytotoxic T cells [letter].** *Nature* 1977, **268**:154-156

52. Gillis S, Baker PE, Ruscetti FW, Smith KA: **Long-term culture of human antigen-specific cytotoxic T cell lines.** *Journal of Experimental Medicine* 1978, **148**:1093-1098

53. Sredni B, Schwartz R: **Antigen-specific, proliferating T lymphocyte clones. Methodology, specificity, MHC restriction and alloreactivity.** *Immunol Rev* 1981, **54**:187-223

54. Allison J, McIntyre B, Bloch D: **Tumor-specific antigen of murine T-lymphoma defined with monoclonal antibody.** *J Immunol* 1982, **129**:2293-2300

55. Meuer SC, Fitzgerald KA, Hussey RE, Hodgdon JC, Schlossman S, Reinherz EL: **Clonotypic structures involved in antigen-specific human T cell function. Relationship to the T3 molecular comlex.** *J Exp Med* 1983, **157**:705-719

56. Meuer SC, Hodgdon JC, Hussey RE, Protentis JP, Schlossman S, Reinherz EL: **Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones.** *J Exp Med* 1983, **158**:988-993

57. Haskins K, Kubo R, Pigeon M, White J, Kappler J, Marrack P: **The major histocompatability compex restricted antigen receptor in T cells. I. Isolation with a monoclonal antibody.** *J Exp Med* 1983, **157**:1149-1158

58. Acuto O, Hussey R, Fitzgerald K, Protentis J, Meuer S, Schlossman S, Reinherz E: **The human T cell receptor: Appearance in ontogeney and biochemical relationship of alpha and beta subunits on IL2-dependent clones and T cell tumors.** *Cell* 1983, **34**:717-726

59. McIntyre B, Allison J: **The mouse T cell receptor: Structural heterogeneity of molecules of normal T cells defined by xenoantiserum.** *Cell* 1983, **34**:739-746

60. Kappler J, Kubo R, Haskins K, White J, Marrack P: **The mouse T cell receptor: Comparison of MHC-restricted recptors on two T cell hybridomas.** *Cell* 1983, **34**:727-737

61. Hedrick S, Cohen D, Nielson E, Davis MM: **Isolation of cDNA clones encoding T cell-specific membrane associated proteins.** *Nature* 1984, **308**:149-155

62. Dennert G: **Cloned lines of natural killer cells.** *Nature* 1980, **287**:47-49

63. Dennert G, Yogeeswaran G, Yamagata S: **Cloned cell lines with natural killer activity. Specificity, function, and cell surface markers.** *J Exp Med* 1981, **153**:545-556

64. Hercend T, Reinherz E, Meuer S, Schlossman S, Ritz J: **Phenotypic and functional heterogeneity of human cloned natural killer cell lines.** *Nature* 1983, **301**:158-160

65. Hercend T, Griffin J, Bensussan A, Schmidt R, Edson M, Brennan A, Murray C, Daley J, Schlossman S, Ritz J: **Generation of monoclonal antibodies to a human natural killer clone. Characterization of two natural killer-associated antigens, NKH1A and NKH2, expressed on subsets of large granular lymphocytes.** *J Clin Invest* 1985, **75**:932-943

66. Gillis S, Ferm MM, Ou W, Smith KA: **T cell growth factor: parameters of production and a quantitative microassay for activity.** *J Immunol* 1978, **120**:2027-2032

67. Smith KA, Lachman LB, Oppenheim JJ, Favata MF: **The functional relationship of the interleukins.** *J Exp Med* 1980, **151**:1551-1556

68. Gery I, Waksman BH: **Potentiation of the T-lymphocyte response to mitogens: The cellular source of potentiating mediators.** *J Exp Med* 1972, **136**:143-155

69. Isakson P, Pure E, Vitteta E, Krammer P: **T cell-derived B cell differentiation factor(s): effeect on the isotype switch of murine B cells.** *J Exp Med* 1982, **155**:734-748

70. Howard M, Farrar J, Hilfiker M, Johnson B, Takatsu K, Hamaoka T, Paul WE: **Identification of a T cell-derived B cell growth factor distinct from interleukin 2.** *J Exp Med* 1982, **155**:914-923

71. Takatsu K, Harada N, Hara Y, Takahama Y, Yamada G, Dobashi K, Hamaoka T: **Purification and physicochemical characterization of murine T cell replacing factor (TRF).** *J Immunol* 1985, **134**:382-389

72. Hirano T, Taga T, Nakano N, Yasukawa K, Kashiwamura S, Shimizu K, Nakajima K, Pyun KH, Kishimoto T: **Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2).** *Proc Natl Acad Sci U S A* 1985, **82**:5490-5494

73. Gately MK, Wilson DE, Wong HL: **Synergy between recombinant interleukin 2 (rIL2) and IL2-depleted lymphokine-containing supernatants in fcilitating allogeneic human cytolytic T lymphocyte responses in vitro.** *J Immunol* 1986, **136**:1274-1282

74. Namen A, Schmierer A, March C, Overell R, Park L, Urdal D, Mochizuki D: **B cell precursor growth-promoting activity. Purification and characterization of a growth factor active on lymphocyte precursors.** *J Exp Med* 1988, **167**:988-1002

75. Vieira P, de Waal-Malefyt R, Dang M-N, Johnson K, Kastelein R, Fiorentino D, de Vries J, Roncarolo M-G, Mosmann T, Moore K: **Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: Homology to Epstein-Barr virus open reading frame BCRF1.** *Proc Natl Acad Sci U S A* 1991, **88**:1172-1176

76. Chan S, Perussia B, Gupta J, Kobayashi M, Pospisil M, Young H, Wolf S, Young D, Clark S, Trinchieri G: **Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers.** *J Exp Med* 1991, **173**:869-879

77. Burton J, Bamford R, Peters C, Grant A, Kurys G, Goldman C, Brennan J, Roessler E, Waldmann T: **A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells.** *Proc Natl Acad Sci U S A* 1994, **91**:4935-4939

78. Grabstein K, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V, Beers C, Richardson J, Schoenborn M, Ahdieh M, *et al*: **Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor.** *Science* 1994, **264**:965-968

79. Schreiber R, Pace J, Russell S, Altman A, Katz D: **Macrophage-activating factor produced by a T cell hybridoma: physiochemical and biosynthetic resemblance to gamma-interferon.** *J Immunol* 1983, **131**:826-832

80. Burgess A, Camakaris J, Metcalf D: **Purification and properties of colony-stimulating factor from mouse lung-conditioned medium.** *J Biol Chem* 1977, **252**:1998-2003

81. Gough N, Gough J, Metcalf D, Kelso A, Grail D, Nicola N, Burgess A, Dunn A: **Molecular cloning of cDNA encoding a murine haematopoietic growth regulator, granulocyte-macrophage colony stimulating factor.** *Nature* 1984, **309**:763-767

82. Naito K, Inaba K, Hirayama Y, Inaba-Miyama M, Sudo T, Muramatsu S: **Macrophage factors which enhance the mixed leukocyte reaction initiated by dendritic cells.** *J Immunol* 1989, **142**:1834-1839

83. Smith KA, Favata MF, Oroszlan S: **Production and characterization of monoclonal antibodies to human interleukin 2: strategy and tactics.** *J Immunol* 1983, **131**:1808-1815

84. Budd R, Smith KA: **Interleukin 2 immunoassay using monoclonal antibodies.** *Biotechnology* 1986, **4**:983-986

85. Openshaw P, Murphy EE, Hosken NA, Maino V, Davis K, Murphy K, O'Garra A: **Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations.** *J Exp Med* 1995, **182**:1357-1367

86. Green S, Dobrjansky A, Carswell E, Kassel RL, Old LJ, Fiore N, MK S: **Partial purification of a serum factor that causes necrosis of tumors.** *Proc Natl Acad Sci USA* 1976, **73**:381-385

87. Beutler B, Cerami A: **Cachectin and tumour necrosis factor as two sides of the same biological coin.** *Nature* 1986, **320**:584-588

88. Tracey K, Beutler B, Lowry S, Merryweather J, Wolpe S, Milsark I, Hariri R, Fahey T, Zentella A, Albert J, *et al*: **Shock and tissue injury induced by recombinant human cachectin.** *Science* 1986, **234**:470-474

89. Dinarello C, Cannon J, Wolff S, Bernheim H, Beutler B, Cerami A, Figari I, Palladino M, O'Connor J: **Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1.** *J Exp Med* 1986, **163**:1433-1450

90. Brennan F, Chantry D, Jackson A, Maini R, Feldman M: **Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis.** *The Lancet* 1989, **2**:244-247

91. Williams R, Feldmann M, Maini R: **Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis.** *Proc Natl Acad Sci U S A* 1992, **89:**9784-9788
92. Elliot M, Maini R, Feldmann M, *et al*: **Repeated therapy with monoclonal antibody to tumor necrosis factor alpha (cA2) in patients with rheumatoid arthritis.** *Lancet* 1994, **344(1):**115-1127
93. Present D, Rutgeerts P, Targan S, *et al*: **Infliximab for the treatment of fistulas in patients with Crohn's disease.** *N Eng J Med* 1999, **340:**1398-1405
94. Mosmann T, Cherwinski H, Bond M, Gieden M, Coffman R: **Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins.** *J Immunol* 1986, **136:**2348-2357
95. Snapper C, Paul W: **Interferon-gamma and B cell stimulatory factor-I reciprocally regulate Ig isotype production.** *Science* 1987, **236:**944-947
96. Cher D, Mosmann T: **Two types of murine helper T cell clone. II. Delayed-type hypresensitivity is mediated by Th-1 clones.** *J Immunol* 1987, **138:**3688-3694
97. Swain S, Weinberg A, English M, Huston G: **IL-4 directs the development of Th2-like helper effectors.** *J Immunol* 1990, **145:**3796-3806
98. Croft M, Duncan DD, Swain SL: **Response of naive antigen-specific CD4+ T cells in vitro: characteristics and antigen-presenting cell requirements.** *J Exp Med* 1992, **176:**1431-1437
99. Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE: **Generation of interleukin 4 (IL4)-producing cells in vivo and in vitro: IL2 and IL4 are required for in vitro generation of IL4-producing cells.** *The J Exp Med* 1990, **172:**921-929
100. Macatonia S, Hsieh C, Murphy K, O'Garra A: **Dendritic cells and macrophages are required for Th1 development of CD4+ T cells from alpha beta TCR transgenic mice: IL-12 substitution for macrophages to stimulate IFN-gamma production is IFN-gamma-dependent.** *Int Immunol* 1993, **5:**1119-1128
101. Macatonia S, Hosken N, Litton M, Vieira P, Hsieh C, Culpepper J, Wysocka M, Trinchieri G, Murphy K, O'Garra A: **Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells.** *J Immunol* 1995, **154:**5071-5079
102. Bucy R, Panoskaltsis-Mortari A, Huang G, Li J, Karr L, Ross M, Russell J, Murphy K, Weaver C: **Heterogeneity of single cell cytokine gene expression in clonal T cell populations.** *J Exp Med* 1994, **180:**1251-1262
103. Szabo S, Kim S, Costa G, Zhang X, Fathman C, Glimcher L: **A novel transcription factor, T-bet, directs Th1 lineage commitment.** *Cell* 2000, **100:**655-669
104. Solymar D, Agarwal S, Bassing C, Alt F, Rao A: **A 3' enhancer in the IL-4 gene regulates cytokine production by Th2 cells and mast cells.** *Immunity* 2002, **17:**41-50
105. Glimcher L, Murphy K: **Lineage commitment in the immune system: the T helper lymphocyte grows up.** *Genes Dev* 2000, **14:**1693-1711
106. Podack ER, Koningsberg PJ: **Cytolytic T cell granules. Isolation, structural, biochemical, and functional characterization.** *J Exp Med* 1984, **160:**695-710
107. Podack ER, Young JD, Cohn ZA: **Isolation and biochemical and functional characterization of peforin 1 from cytolytic T cell granules.** *Proc Natl Acad Sci USA* 1985, **82:**8629-8633
108. Walter E, Greenberg P, Gilbert M, Finch R, Watanabe K, Thomas E, Riddell S: **Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor.** *N Engl J Med* 1995, **333:**1038-1044
109. O'Reilly R, Small T, Papadopoulos E, Lucas K, Lacerda J, Koulova L: **Biology and adoptive cell therapy of Epstein-Barr virus-associated lymphoproliferative disorders in recipients of marrow allografts.** *Immunol Rev* 1997, **157:**195-216
110. Yee C, Thompson J, Roche P, Byrd D, Lee P, Piepkorn M, Kenyon K, Davis M, Riddell S, Greenberg P: **Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of t cell-mediated vitiligo.** *J Exp Med* 2000, **192:**1637-1644
111. Dudley M, Wunderlich J, Robbins P, Yang J, Hwu P, Schwartzentruber D, Topalian S, Sherry R, Restifo N, Hubicki A, Robinson M, Raffeld M, Duray P, Seipp C, Rogers-Freezer L, Morton K, Mavroukakis S, White D, Rosenberg S: **Cancer Regression and Autoimmunity in Patients After Clonal Repopulation with Antitumor Lym-**

phocytes [epub ahead of print] [Record Supplied By Publisher]. *Science* 2002, **10.**1126/science.1076514:
112. Yee C, Riddell S, Greenberg P: **In vivo tracking of tumor-specific T cells.** *Curr Opin Immunol* 2001, **13:**141-146
113. Taniguchi T, Matsui H, Fujita T, Takaoka C, Kashima N, Yoshimoto R, Hamuro J: **Structure and expression of a cloned cDNA for human interleukin-2.** *Nature* 1983, **302:**305-310
114. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, Matory YL, Skibber JM, Shiloni E, Vetto JT, *et al*: **Observations on the systemic administration of autologous lymphokine – activated killer cells and recombinant interleukin-2 to patients with metastatic cancer.** *N Engl J Med* 1985, **313:**1485-1492
115. Rosenberg SA: **Perspectives on the use of interleukin 2 in cancer treatment.** *The Cancer Journal* 1997, **3(Supplement 1):**S2-S6
116. Cantrell DA, Smith KA: **The interleukin-2 T-cell system: a new cell growth model.** *Science* 1984, **224:**1312-1316
117. Smith KA: **Rational interleukin 2 therapy.** *Cancer Journal* 1997, **3:**S137-140
118. Smith K: **Interleukin 2 Immunotherapy.** *In: Therapeutic Immunology (Edited by: Austen F, Burakoff S, Rosen F, Strom T) Malden, MA: Blackwell Science, Inc.* 2001, 240-250
119. Robb RJ, Munck A, Smith KA: **T cell growth factor receptors. Quantitation, specificity, and biological relevance.** *J Exp Med* 1981, **154:**1455-1474
120. Leonard WJ, Depper JM, Uchiyama T, Smith KA, Waldmann TA, Greene WC: **A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor.** *Nature* 1982, **300:**267-269
121. Sharon M, Klausner RD, Cullen BR, Chizzonite R, Leonard WJ: **Novel interleukin-2 receptor subunit detected by cross-linking under high affinity conditions.** *Science* 1986, **234:**859-863
122. Teshigawara K, Wang HM, Kato K, Smith KA: **Interleukin 2 high-affinity receptor expression requires two distinct binding proteins.** *J Exp Med* 1987, **165:**223-238
123. Takeshita T, Ohtani K, Asao H, Kumaki S, Nakamura M, Sugamura K: **An associated molecule, p64, with IL-2 receptor beta chain. Its possible involvement in the formation of the functional intermediate – affinity IL-2 receptor complex.** *J Immunol* 1992, **148:**2154-2158
124. Waldmann TA, O'Shea J: **The use of antibodies against the IL-2 receptor in transplantation.** *Curr Opin Immunol* 1998, **10:**507-512
125. Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, Neylan J, Wilkinson A, Ekberg H, Gaston R, Backman L, Burdick J: **Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group.** *N Engl J Med* 1998, **338:**161-165
126. Beniaminovitz A, Itescu S, Lietz K, Donovan M, Burke E, Groff B, Edwards N, Mancini D: **Prevention of rejection in cardiac transplantation by blockade of the interleukin-2 receptor with a monoclonal antibody.** *N Engl J Med* 2000, **342:**613-619
127. Moreland L, Baumgartner S, Schiff M, *et al*: **Treatment of rheumatoid arthritis with a recombinant tumor necrosis factor receptor (p75)-Fc fusion protein.** *N Eng J Med* 1997, **337:**141-147
128. Takeshita T, Asao H, Ohtani K, Ishii N, Kumaki S, Tanaka N, Manukata H, Nakamura M, Sugamura K: **Cloning of the Gamma chain of the Human IL2 receptor.** *Science* 1992, **257:**379-382
129. Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, McBride OW, Leonard WJ: **Interleukin 2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans.** *Cell* 1993, **73:**147-157
130. Leonard W, Shores E, Love P: **Role of the common cytokine receptor gamma chain in cytokine signaling and lymphoid development.** *Immunol Rev* 1995, **148:**97-114
131. Cavazzana-Calvo M, Hacein-Bey S, de SBG, Gross F, Yvon E, Nusbaum F, Selz F, Hue C, Certain S, Casanova J, Bousso P, Deist F, Fischer A: **Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease.** *Science* 2000, **288:**669-672
132. Gillis S, Crabtree GR, Smith KA: **Glucocorticoid-induced inhibition of T cell growth factor production. I. The effect on mitogen-induced lymphocyte proliferation.** *J Immunol* 1979, **123:**1624-1631
133. Yang-Yen H-F, Chambard J-C, Sun Y-L, Smeal T, Schmidt TJ, Drouin J, Karin M: **Transcriptional inteference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding**

due to direct protein-protein interaction. *Cell* 1990, **62**:1205-1215

134. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M: **Immunosupression by glucocorticoids: Inhibition of NFkB activity through induction of IkB synthesis.** *Science* 1995, **270**:286-290

135. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS: **Role of transcriptional activation of IkB-alpha in mediation of immunosupression by glucocorticoids.** *Science* 1995, **270**:283-286

136. Bunjes D, Hardt C, Rollinghoff M, Wagner H: **Cyclosporin A mediates immunosuppression of primary cytotoxic T cell responses by impairing the release of interleukin 1 and interleukin 2.** *Eur J Immunol* 1981, **11**:657-661

137. Northrop JP, Ho SN, Chen L, Thomas DJ, Timmerman LA, Nolan GP, Admon A, Crabtree GR: **NF-AT components define a family of transcription factors targeted in T ell activation.** *Nature* 1994, **369**:497-502

138. Grigoriadis G, Zhan Y, Grumont R, Metcalf D, Handman E, Cheers C, Gerondakis S: **The Rel subunit of NF-kappaB-like transcription factors is a positive and negative regulator of macrophage gene expression: distinct roles for Rel in different macrophage populations.** *EMBO J* 1996, **15**:7099-7107

139. Tumang J, Owyang A, Andjelic S, Jin Z, Hardy R, Liou M, Liou H: **c-Rel is essential for B lymphocyte survival and cell cycle progression.** *Eur J Immunol* 1998, **28**:4299-4312

140. Liou H, Jin Z, Tumang J, Andjelic S, Smith K, Liou M: **c-Rel is crucial for lymphocyte proliferation but dispensable for T cell effector function.** *Int Immunol* 1999, **11**:361-371

141. Hsia C, Cheng S, Owyang A, Dowdy S, Liou H: **c-Rel regulation of the cell cycle in primary mouse B lymphocytes [In Process Citation].** *Int Immunol* 2002, **14**:905-916

142. Grumont R, Rourke I, O'Reilly L, Strasser A, Miyake K, Sha W, Gerondakis S: **B lymphocytes differentially use the Rel and nuclear factor kappaB1 (NF-kappaB1) transcription factors to regulate cell cycle progression and apoptosis in quiescent and mitogen-activated cells.** *J Exp Med* 1998, **187**:663-674

143. Sen R, Baltimore D: **Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism.** *Cell* 1986, **47**:921-928

144. Shapiro A, Lakey J, Ryan E, Korbutt G, Toth E, Warnock G, Kneteman N, Rajotte R: **Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen.** *N Engl J Med* 2000, **343**:230-238

145. Fu X, Kessler D, Veals S, Levy D, Darnell J: **ISGF3, the transcriptional activator induced by interferon alpha, consists of multiple interacting polypeptide chains.** *Proc Natl Acad Sci U S A* 1990, **87**:8555-8559

146. Darnell J, Kerr I, Stark G: **Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins.** *Science* 1994, **264**:1415-1421

147. Brivanlou A, Darnell J: **Signal transduction and the control of gene expression.** *Science* 2002, **295**:813-818

148. O'Shea J, Gadina M, Schreiber R: **Cytokine signaling in 2002: new surprises in the Jak/Stat pathway.** *Cell* 2002, **109(Suppl)**:S121-131

149. Witthuhn B, Quelle F, Silvennoinen O, Yi T, Tang B, Miura O, Ihle J: **JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin.** *Cell* 1993, **74**:227-236

150. Ihle J: **The Janus protein tyrosine kinase family and its role in cytokine signaling.** *Adv Immunol* 1995, **60**:1-35

151. O'Shea J, Visconti R, Cheng T, Gadina M: **Jaks and stats as therapeutic targets.** *Ann Rheum Dis* 2000, **59(Suppl 1)**:i115-118

152. Schlossman S, Ben-Efraim S, Yaron A, Sober H: **Immunochemical studies on the antigenic determinants required to elicit delayed and immediate hypersensitivity reactions.** *J Exp Med* 1966, **123**:1083-1095

153. Unanue E, Askonas BA: **Persistence of immunogenicity of antigen after uptake by macrophages.** *J Exp Med* 1968, **127**:

154. Zeigler HK, Unanue ER: **Decrease in macrophage antigen catabolism caused by ammonia and chloroquin is associated with inhibition of antigen presentation to T cells.** *Proc Natl Acad Sci USA* 1982, **79**:175-178

155. Mackaness G: **The influence of immunologically committed lymphoid cells on macrophage activity in vivo.** *J Exp Med* 1969, **129**:973-992

156. Nathan C, Karnovsky M, David J: **Alterations of macrophage functions by mediators from lymphocytes.** *J Exp Med* 1971, **133**:1356-1376

157. Nathan C, Remold H, David J: **Characterization of a lymphocyte factor which alters macrophage functions.** *J Exp Med* 1973, **137**:275-290

158. Lindahl P, Gresser I, Leary P, Tovey M: **Enhanced expression of histocompatibility antigens of lymphoid cells in mice treated with interferon.** *J Infect Dis* 1976, **133(Suppl)**:A66-68

159. Vignaux F, Gresser I: **Differential effects of interferon on the expression of H-2K, H-2D, and Ia antigens on mouse lymphocytes.** *J Immunol* 1977, **118**:721-723

160. Nathan C, Murray H, Wiebe M, Rubin B: **Identification of interferon gamma as the lymphokne that activates human macrophage oxidative metabolism and antimicrobial activity.** *J Exp Med* 1983, **158**:670-689

161. Murray H, Rubin B, Rothermal C: **Killing of intracellular Leishmania donovani by lymphokine-stimulated human mononuclear phagocytes: evidence that interferon-gamma is the activating lymphokine.** *J Clin Invest* 1983, **72**:1506-1510

162. Babbitt B, Allen P, Matsueda G, Haber E, Unanue E: **Binding of immunogenic peptides to Ia histocompatibility molecules.** *Nature* 1985, **317**:359-361

163. Bjorkman PJ, Saper MA, Samaoui B, Bennett WS, Strominger JL, Wiley DC: **Structure of the human class I histocompatability antigen, HLA-A2.** *Nature* 1987, **329**:506-511

164. Davis M, Bjorkman P: **T cell antigen receptor genes and T cell recognition.** *Nature* 1988, **334**:395-402

165. Reinherz EL, Schlossman SF: **The differentiation and function of T lymphocytes.** *Cell* 1980, **4**:821-827

166. Scollay R, Bartlett P, Shortman K: **T cell development in the adult murine thymus: changes in the expression of the surface antigens Ly2, L3T4 and B2A2 during development from early precursor cells to emigrants.** *Immunol Rev* 1984, **82**:79-103

167. Scollay R, Shortman K: **Identification of early stages of T lymphocyte development in the thymus cortex and medulla.** *J Immunol* 1985, **134**:3632-3642

168. Kruisbeek A, Fultz M, Sharrow S, Singer A, Mond J: **Early development of the T cell repertoire. In vivo treatment of neonatal mice with anti-Ia antibodies interferes with differentiation of I-restricted T cells but not K/D-restricted T cells.** *J Exp Med* 1983, **157**:1932-1946

169. Kruisbeek A, Mond J, Fowlkes B, Carmen J, Bridges S, Longo D: **Absence of the Lyt-2-, L3T4+ lineage of T cells in mice treated neonatally with anti-I-A correlates with absence of intrathymic I-A-bearing antigen-presenting cell function.** *J Exp Med* 1985, **161**:1029-1047

170. Kisielow P, Teh H, Bluthmann H, van Boehmer H: **Positive selection of antigen-specific T cells in thymus by restricting MHC molecules.** *Nature* 1988, **335**:730-733

171. Teh H, Kisielow P, Scott B, Kishi H, Uematsu Y, Bluthmann H, van Boehmer H: **Thymic major histocompatibility complex antigens and the alpha beta T-cell receptor determine the CD4/CD8 phenotype of T cells.** *Nature* 1988, **335**:229-233

172. Kisielow P, Bluthmann H, Staerz U, Steinmetz M, van Boehmer H: **Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4+8+ thymocytes.** *Nature* 1988, **333**:742-746

173. Oettinger M, Schatz D, Gorka C, Baltimore D: **RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination.** *Science* 1990, **248**:1517-1523

174. Namen A, Lupton S, Hjerrild K, Wignall J, Mochizuki D, Schmierer A, Mosley B, March C, Urdal D, Gillis S: **Stimulation of B-cell progenitors by cloned murine interleukin-7.** *Nature* 1988, **333**:571-573

175. Mackall C, Fry T, Bare C, Morgan P, Galbraith A, Gress R: **IL-7 increases both thymic-dependent and thymic-independent T-cell regeneration after bone marrow transplantation.** *Blood* 2001, **97**:1491-1497

176. Fry T, Christensen B, Komschlies K, Gress R, Mackall C: **Interleukin-7 restores immunity in athymic T-cell-depleted hosts.** *Blood* 2001, **97**:1525-1533

177. Fry T, Mackall C: **Interleukin-7: from bench to clinic.** *Blood* 2002, **99**:3892-3904

178. Caux C, Dezutter-Dambuyant C, Schmitt D, Bancherau J: **GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhan's cells.** *Nature* 1992, **360:**258-261

179. Inaba K, Steinman R, Pack M, Aya H, Inaba T, Sudoo S, Wolpe S, Schuler G: **Identification of proliferating dendritic cells in mouse blood.** *J Exp Med* 1992, **175:**1157-1167

180. Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch P, Steinman R, Schuler G: **Proliferating dendritic cell progenitors in human blood.** *J Exp Med* 1994, **180:**83-93

181. Sallusto F, Lanzavecchia A: **Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha.** *J Exp Med* 1994, **179:**1109-1118

182. Sallusto F, Cella M, Danieli C, Lanzavecchia A: **Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products.** *J Exp Med* 1995, **182:**389-400

183. Winzler C, Rovere P, Rescigno M, Granucci F, Penna G, Adorini L, Zimmermann V, Davoust J, Ricciardi-Castagnoli P: **Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures.** *J Exp Med* 1997, **185:**317-328

184. Maurer T, Heit A, Hochrein H, Ampenberger F, O'Keeffe M, Bauer S, Lipford G, Vabulas R, Wagner H: **CpG-DNA aided cross-presentation of soluble antigens by dendritic cells [In Process Citation].** *Eur J Immunol* 2002, **32:**2356-2364

185. Buelens C, Verhasselt V, De GD, Thielemans K, Goldman M, Willems F: **Human dendritic cell responses to lipopolysaccharide and CD40 ligation are differentially regulated by interleukin-10.** *Eur J Immunol* 1997, **27:**1848-1852

186. Siegal F, Kadowaki N, Shodell S, Fitzgerald-Bocarsly P, Shah K, Ho S, Antonenko S, Liu Y-j: **The nature of the principle interferon-producing cells in human blood.** *Science* 1999, 1835-1837

187. Bancherau J, Schuler-Thurner B, Palucka A, Schuler G: **Dendritic cells as vectors for therapy.** *Cell* 2001, **106:**271-274

188. Berger T, Feuerstein B, Strasser E, Hirsch U, Schreiner D, Schuler G, Schuler-Thurner B: **Large-scale generation of mature monocyte-derived dendritic cells for clinical application in cell factories [In Process Citation].** *J Immunol Methods* 2002, **268:**131

189. Bancherau J, de Paoli P, Valle A, Garcia E, Rousset F: **Long-term human B cell lines dependent on interleukin-4 and antibody to CD40.** *Science* 1991, **251:**70-72

190. Bancherau J, Rouset F: **Growing human B lymphocytes in the CD40 system.** *Nature* 1991, **353:**678-679

191. Lederman S, Yellin M, Krichevsky A, Belko J, Lee J, Chess L: **Identification of a novel surface protein on activated CD4+ T cells that induces contact-dependent B cell differentiation (help).** *J Exp Med* 1992, **175:**1091-1099

192. Lederman S, Yellin MJ, Inghirami G, Lee JJ, Knowels DM, Chess L: **Molecular interactions mediating T-B lymphocyte collaboration in human lymphoid follicles. Roles of T cell-B cell activating molecules (5c8 antigen) and CD40 in contact-dependent help.** *J Immunol* 1992, **149:**3817-3826

193. Fuleihan R, Ramesh N, Loh R, Jabara H, Rosen R, Chatila T, Fu S, Stamenkovic I, Geha R: **Defective expression of the CD40 ligand in X chromosome-linked immunoglobulin deficiency with normal or elevated IgM.** *Proc Natl Acad Sci U S A* 1993, **90:**2170-2173

194. Papavasiliou F, Schatz D: **Somatic hypermutation of immunoglobulin genes: merging mechanisms for genetic diversity.** *Cell* 2002, **109(suppl):**35-44

195. Stavnezer J: **Antibody class switching.** *Adv immunol* 1996, **61:**79-146

196. Zan H, Cerutti A, Dramitinos P, Schaffer A, Li Z, Casali P: **Induction of Ig somatic hypermutation and class switching in a human monoclonal IgM+ IgD+ B cell line in vitro: definition of the requirements and modalities of hypermutation.** *J Immunol* 1999, **162:**3437-3447

197. Cerutti A, Zan H, Schaffer A, Bergsagel L, Harindranath N, Max E, Casali P: **CD40 ligand and appropriate cytokines induce switching to IgG, IgA, and IgE and coordinated germinal center and plasmacytoid phenotypic differentiation in a human monoclonal IgM+IgD+ B cell line.** *J Immunol* 1998, **160:**2145-2157

198. Litinskiy M, Nardelli B, Hilbert D, He B, Schaffer A, Casali P, Cerutti A: **DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL.** *Nat Immunol* 2002, **3:**822-829

199. Balazs M, Martin F, Zhou T, Kearney J: **Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses.** *Immunity* 2002, **17:**341-352

200. Poltorak A, He X, Smirnova I, Liu M, Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B: **Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene.** *Science* 1998, **282:**2085-2088

201. Carswell E, Old L, Kassel R, Green S, Fiore N, Williamson B: **An endotoxin-induced serum factor that causes necrosis of tumors.** *Proc Natl Acad Sci U S A* 1975, **72:**3666-3670

202. Kawakami M, Cerami A: **Studies of endotoxin-induced decrease in lipoprotein lipase activity.** *J Exp Med* 1981, **154:**631-639

203. Pennica D, Hayflick J, Bringman T, Palladino M, Goeddel D: **Cloning and expression in Escherichia coli of the cDNA for murine tumor necrosis factor.** *Proc Natl Acad Sci U S A* 1985, **82:**6060-6064

204. Beutler B, Mahoney J, Le TN, Pekala P, Cerami A: **Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells.** *J Exp Med* 1985, **161:**984-995

205. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S: **A Toll-like receptor recognizes bacterial DNA.** *Nature* 2000, **408:**740-745

206. Alexopoulou L, Holt A, Medzhitov R, Flavell R: **Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3.** *Nature* 2001, **413:**732-738

207. Garboczi D, Ghosh P, Utz U, Fan Q, Biddeson W, Wily D: **Structure of the complex between human T cell receptor, viral peptide and HLA-A2.** *Nature* 1996, **384:**134-141

208. Garcia K, Degano M, Stanfield R, Brumark A, Jackson M, Peterson P, Teyton L, Wilson I: **An alpha/beta T cell recptor structure at 2.5 A and its orientation in the TCR-MHC complex.** *Science* 1996, **274:**209-219

209. Reinherz E, Tan K, Tang L, Kern P, Liu J, Xiong Y, Hussey R, Smolyar A, Hare B, Zhang R, *et al*: **The crystal structure of a T cell recptor in complex with peptide and MHC Class II.** *Science* 1999, **286:**1913-1921

210. Wang J-H, Reinherz E: **Structural basis of T cell recognition of peptides bound to MHC molecules.** *Mol Immunol* 2002, **38:**1039-1049

211. Altman JD, Moss PAH, Goulder PJR, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM: **Phenotypic analysis of antigen-specific T lymphocytes.** *Science* 1996, **274:**94-96

212. Kern F, Surel I, Brock C, Freistedt B, Radtke H, Scheffold A, Blasczyk R, Reinke P, Schneider-Mergener J, Radbruch A, Walden P, Volk H: **T-cell epitope mapping by flow cytometry.** *Nat Med* 1998, **4:**975-978

213. Kern F, Faulhaber N, Frommel C, Khatamzas E, Prosch S, Schonemann C, Kretzschmar I, Volkmer-Engert R, Volk H, Reinke P: **Analysis of CD8 T cell reactivity to cytomegalovirus using protein-spanning pools of overlapping pentadecapeptides.** *Eur J Immunol* 2000, **30:**1676-1682

214. Maecker H, Dunn H, Suni M, Khatamzas E, Pitcher C, Bunde T, Persaud N, Trigona W, Fu T, Sinclair E, Bredt B, McCune J, Maino V, Kern F, Picker L: **Use of overlapping peptide mixtures as antigens for cytokine flow cytometry.** *J Immunol Methods* 2001, **255:**27-40

215. Grakoui A, Bromley S, Sumen C, Davis M, Shaw A, Allen P, Dustin M: **The immunological synapse: a molecular machine controlling T cell activation.** *Science* 1999, **285:**221-227

216. Sylvestre D, Ravetch J: **Fc receptors initiate the Arthus reaction: redefining the inflammatory cascade.** *Science* 1994, **265:**1095-1098

217. Sylvestre D, Ravetch J: **A dominant role for mast cell Fc receptors in the Arthus reaction.** *Immunity* 1996, **5:**387-390

218. Sylvestre D, Clynes R, Ma M, Warren H, Carroll M, Ravetch J: **Immunoglobulin G-mediated inflammatory responses develop normally in complement-deficient mice.** *J Exp Med* 1996, **184:**2385-2392

219. Ravetch J, Clynes R: **Divergent roles for Fc receptors and complement in vivo.** *Annu Rev Immunol* 1998, **16:**421-432

220. Ujike A, Ishikawa Y, Ono M, Yuasa T, Yoshino T, Fukumoto M, Ravetch J, Takai T: **Modulation of immunoglobulin (Ig)E-medi-**

ated systemic anaphylaxis by low-affinity Fc receptors for IgG. *J Exp Med* 1999, **189:**1573-1579

221. Clynes R, Maizes J, Guinamard R, Ono M, Takai T, Ravetch J: **Modulation of immune complex-induced inflammation in vivo by the coordinate expression of activation and inhibitory Fc receptors.** *J Exp Med* 1999, **189:**179-185

222. Cochran B, Reffel A, Stiles C: **Molecular cloning of a set of genes sequences regulated by platelet-derived growth factor.** *Cell* 1983, **33:**939-947

223. Beadling C, Johnson KW, Smith KA: **Isolation of interleukin 2-induced immediate-early genes.** *Proc Natl Acad Sci U S A* 1993, **90:**2719-2723

224. Herbolt S, Chastagner P, Samady L, Moreau J-L, Demaison C, Froussard P, Liu X, Bonnet J, Theze J: **IL2-dependent expression of genes involved in cytoskeleton organization, oncogene regulation and transcriptional control.** *J Immunol* 1999, **162:**3280-3288

225. Ehlers S, Benini J, Held H, Roeck C, Alber G, Uhlig S: **Alpha-beta T cell receptor-positive cells and interferon-gamma, but not inducible nitric oxide synthase, are critical for granuloma necrosis in a mouse model of mycobacteria-induced pulmonary immunopathology.** *J Exp Med* 2001, **194:**1847-1859

226. Paoletti E, Tartaglia J, Taylor J: **Safe and effective poxvirus vectors – NYVAC and ALVAC.** *Dev Biol Stand* 1994, **82:**65-69

227. Plotkin S, Cadoz M, Meignier B, Meric C, Leroy O, Excler J, Tartaglia J, Paoletti E, Gonczol E, Chappuis G: **The safety and use of canarypox vectored vaccines.** *Dev Biol Stand* 1995, **84:**165-170

228. Ferrari G, Berend C, Ottinger J, Dodge R, Bartlett J, Toso J, Moody D, Tartaglia J, Cox W, Paoletti E, Weinhold K: **Replication-defective canarypox (ALVAC) vectors effectively activate anti-human immunodeficiency virus-1 cytotoxic T lymphocytes present in infected patients: implications for antigen-specific immunotherapy.** *Blood* 1997, **90:**2406-2416

229. Clements-Mann M, Weinhold K, Matthews T, Graham B, Gorse G, Keefer M, McElrath M, Hsieh R, Mestecky J, Zolla-Pazner S, Mascola J, Schwartz D, Siliciano R, Corey L, Wright P, Belshe R, Dolin R, Jackson S, Xu S, Fast P, Walker M, Stablein D, Excler J, Tartaglia J, Paoletti E, *et al*: **Immune responses to human immunodeficiency virus (HIV) type 1 induced by canarypox expressing HIV-1MN gp120, HIV-1SF2 recombinant gp120, or both vaccines in seronegative adults. NIAID AIDS Vaccine Evaluation Group.** *J Infect Dis* 1998, **177:**1230-1246