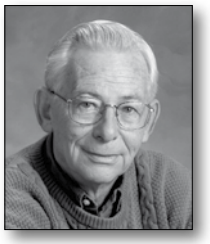

Let's Get Something Straight

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Immunologists: What Are These People Talking About?

My first exposure to an immunologist was in a graduate school course, about 1963. The then-young man who taught the course was a physician, William Arndt. In addition to his scientific acumen, he was friendly, charming, handsome, and enthusiastic. Others said he was knowledgeable, perhaps even a young genius. I had to take their word for it because I had no idea what he was talking about. His vocabulary seemed to be comprised only of words I had never heard and which I could not find in the dictionaries I had. What is more, I knew of no other student who knew what Dr Arndt was talking about. I think I passed the course, but I must have made lucky guesses on "True or False?" tests.

I recently asked a friend of mine, an immunologist (I try to be inclusive), why immunologists use words no one else understands. He said, probably as a joke, but maybe not, "It provides job security." He went on to say that once others begin to understand their terminology, immunologists change the words so that no one else will understand them; I believe him. What was monocyte chemoattractant protein 1 (or monocyte chemotactic protein 1, or macrophage chemoattractant protein 1), abbreviated MCP-1, now is called CCL2; macrophage inflammatory protein 1 alpha is now CCL3; macrophage inflammatory protein 1 beta is now CCL4; Regulated upon Activation, Normal T-cell Expressed and Secreted or "RANTES" is now CCL5; Macrophage Inflammatory Protein-2 (MIP-2) is now CXCKL2, etc. Much more clear now, right?

There are so many publications alluding to cytokines, chemokines, T-cells, natural killer cells, helper T cells (the Th1 cells and Th2 cells), cytotoxic T lymphocytes, regulatory T cells (of which there seem to be at least three subsets), dendritic cells (also several subsets), Granulocyte-monocyte colony stimulating factor, interleukin, interferons, tumor growth factor, tumor necrosis factor, and such, that I considered it best, drink in hand, to learn some of these words before these people change them again. I do not like to not understand what I am reading. I have had enough trouble with "Beowulf", and "Tristram Shandy," not to speak of papers on quantum mechanics, to last a lifetime. My psychological well-being, my professional skills and, hence, my income are not dependent on my understanding of any of these "classics" but it would be comforting if I did understand them, and I might feel like a better person if

I knew what the hell James Joyce was trying to tell me; I expect that even if I live to be hundred I will not understand "Ulysses." Indeed, if I do not try to understand "Ulysses," I have a better chance at living to be hundred.

Immunology is a field I should know, or at least be conversant in, or at the very least appreciate. I am a virologist and it is obvious that an understanding of immunology is important if I intend to understand the way viruses affect us, protect us, or kill us. Of course, the same can be said for bacteria, parasites, and fungi. The latter could be fodder for another column, but I doubt it.

Therefore, I have been making an effort to understand the language I prefer to call "immunology-speak". Here is what I have learned:

Antibodies (soluble glycoproteins) to various protein components of viruses (please forgive me if I use the word "virus" as a surrogate for "bacterium," "parasite," or "fungus" throughout this article) are produced by immunocompetent (non-immunocompromised) vertebrates in response to infection or contact with the virus (antigen, ie, antibody generator), viral proteins are the antigens and antibodies are anti-antigens. Viruses have many different proteins that can serve as antigens, each of which can stimulate production of antibodies to them. An antibody is a Y-shaped protein used by the immune system to identify and deactivate (neutralize or immobilize) foreign matter, such as viruses. Each antibody recognizes a specific antigen and is unique to its target ("like a lock and key," the lock being the antibody paratope and the key being the antigen epitope).

Immunoglobulins (the word "immunoglobulin" usually means about the same as the word "antibody," except that the immunoglobulin family comprises antibodies as well as receptors for immune proteins, such as cytokines, chemokines, etc. – see below) are antibodies that bind specifically to one or a few closely related antigens or other biochemical agents. Each immunoglobulin binds to a specific epitope. The function of such binding is to protect the host, which it does (we hope) by destroying the antigen or by holding onto it until help arrives. Often, however, the binding of an antibody to an antigen has no direct biological effect. Instead, the major biological effects are consequences of secondary "effector functions" of antibodies, functions mediated by immunoglobulins. The ability to carry out a particular effector function usually requires the antibody to bind to its antigen, but not every immunoglobulin will mediate all effector functions. Such effector functions include fixation of complement (resulting in lysis of cells and release of bioactive molecules) and binding to various cell types, including phagocytic cells, lymphocytes, platelets, mast cells, and basophils, each of which has receptors that bind immunoglobulins. This binding can activate the cells and cause them to do something useful.

Immunoglobulin M (IgM) is the "first responder," produced very soon after exposure to the antigen. It is a large molecule and does not cross various tissue barriers so, for example, finding IgM antibody in the blood of a newborn indicates *in utero* infection, rather than transplacental transfer of antibody from the mother. The latter is achieved by the binding of other immunoglobulins to receptors on trophoblasts in the placenta, resulting in transfer of that immunoglobulin across the placenta. As a result, the transferred maternal immunoglobulin G (IgG) antibody provides immunity to the fetus and to the newborn.

Immunoglobulin M antibody usually persists for no more than a few months. Immunoglobulin G antibody, a smaller molecule, is produced later in infection, and IgG antibody can be detected for the life of the infected individual. Actually, the half-life of IgG antibody is brief, perhaps a few weeks, but the memory B cells that produce it persist and it is the anamnestic response of these cells that allow us to respond appropriately

to re-exposure. Immunoglobulin G, therefore, is the antibody that keeps us from being re-infected with viruses. Bad enough that we continue to have bouts of the "common cold" (caused by numerous viruses, bacteria, and even non-infectious agents), at least we are not susceptible to smallpox (Variola), Measles, Mumps, Rubella, or West Nile viruses after a first exposure to them or after having been vaccinated with (against) them. Were vertebrates repeatedly susceptible, I would not be writing this and you would not be reading it. Also, what would be the evolutionary use of an antibody that is not helpful in this way? In addition to IgM and IgG (four subclasses), there are IgA (two subclasses; the primary function of IgA is to prevent antigens of bacteria, viruses, and food, from crossing various mucosal barriers), IgD (considered by most as the B cell receptor in that it defines specificity of that cell, binds to antigen sometimes T cell independently but usually binds antigen presented by helper T cells), and IgE (as a consequence of its binding to basophils and mast cells, IgE is involved in allergic reactions). Binding of an allergen to IgE on the cells results in the release of various pharmacological mediators, which in turn result in allergic symptoms.

When attached to the surface of B cells (lymphocytes that play a role in the humoral immune response, as opposed to the cell-mediated immune response governed by T cells), the membrane-bound form of the immunoglobulin is sometimes referred to as the B cell receptor; IgD has a transmembrane domain and is inserted into the cell plasma membrane, whereas the other Igs are secreted. Soluble antibodies are found in the blood and tissue fluids, as well as in many secretions. They are synthesized and secreted by plasma cells derived from B cells. Membrane-bound immunoglobulins are only found on the surface of B cells and facilitate the activation and clonal selection of these cells following binding of the antigen, to which they are specific. They subsequently differentiate into plasma cells for antibody generation, or into memory cells that will remember the foreign antigen, should exposure to it occur in the future. In most cases, interaction of the B cell with a T-helper cell is necessary to produce full activation of the B cell and, therefore, for generation of high-titer antibody after antigen binding.

Unfortunately for students, and fortunately for creatures that produce antibody, the overall immune response is a great deal more complex than producing antibody and carrying out the resulting garbage, and most of this complexity arises in antigen presentation (antigen presenting cells) and processing (T-helper cells). Unfortunately, because immunology is still a developing field, new cell subsets and functions create a constantly shifting landscape of language and functional distinctions – some of which turn out to be wrong, or as in the case of T-regulatory cells (Treg), are thought to be wrong and found to be correct, or sometimes only mostly correct.

The central problem that the immune system must face is how to respond to the right things and kill them without killing the whole body, of which it is certainly capable, as attested to by old or recent failures of immune boosters in clinical trials. The approach is 3-fold – generate a very, very large number of recognition receptors that try to occupy all possible physical structures that the body will encounter (generation of receptor diversity), kill those cells that will respond too well to the body structures (positive and negative selection in the thymus of T cells, Major Histocompatibility Complex (MHC) restriction of T cell activation), and have a core group of Treg cells that both recognize inappropriate responses and terminate them. This is the reason every large response needs to be and is verified by T-helper cells; T cells are the only ones that have been "educated" to respond the right way and to avoid the wrong way. While there are certainly T-independent antigens (which bind directly to B cell receptors) and

responses outside T cell control (innate immune responses), every large, specific, and sensitive response is controlled by T cells, and failures of the system (allergies, rejection of the fetus, failure to respond, superantigens, etc.) can be seen as failures of T cells and/or their educational or regulatory processes.

Cells derived from monocytes are responsible for both the early parts of the immune response (antigen recognition and presentation) and the clean-up stages (phagocytosis of garbage). These cells are the first responders to a site of injury or insult, and mediate the transition of an innate immune response (cells become activated, move to the site of insult, and dump cytotoxic substances into the environment, killing themselves and, hopefully, the invader as well) to an acquired immune response (T-helper cells receive and process antigen for presentation to either cytotoxic T cells to kill cells infected with viruses or intracellular bacteria, or B cells to produce antibodies, usually both).

The most effective antigen presenting cell is the dendritic cell (DC) – usually called "a (or the) professional antigen presenting cell." These cells recognize sites of insult or injury (some call them "Danger Signals," others just refer to them as recognition of non-self antigens) and ingest (phagocytose or endocytose) what is in their environment, and then activate local cells or migrate to lymph nodes where they can present those antigens to T-helper cells and initiate a more specific and effective response through cytotoxic T lymphocytes or antibody production by B cells.

However, these cells also activate or prime Treg cells, so that the response can terminate at the appropriate time, killing the invader or dealing with the injury, and then stopping before the body is inappropriately attacked. At this time, one of the largest areas of interest in immunology is determining the importance of these cells – their mechanisms of action, how they succeed, how they fail, and how or if they can be used to make vaccines and treatments more effective.

While innate immune cells, cytotoxic lymphocytes have, and Treg cells likely have, contact-dependent mechanisms of action, many of their effects are mediated at a distance, so let's continue with cytokines.

IFN-alpha and IFN-beta, also known as Type I interferons (IFN-gamma is Type II interferon), were the first of these soluble factors to be described, identified by Isaacs and Lindenmann in 1957 as a factor obtained from virus-infected chicken chorioallantoic membranes and which "interfered" with subsequent viral replication in uninfected cultures. The first of the lymphocyte-derived mediators was described in 1965, when Wheelock reported that phytohemagglutinin induced an interferon-like virus inhibitory substance in leukocyte cultures.

For many immunologists, migration inhibition factor was the first of what came to be known as lymphokines. This was an activity in supernatant fluids from antigen-activated lymphocytes that inhibited the movement of macrophages in *in vitro* assays. It was identified independently and simultaneously in 1966 by David and Bloom. The next of the lymphocyte-derived factors to be described was lymphotoxin (Ruddle and Waksman). The discovery of others followed and, in 1969, Dumonde proposed the term "lymphokine" to describe these factors. Subsequently, activities derived from macrophages and monocytes in culture were called "monokines." These lymphokines and monokines were first described in antigen- or mitogen-activated cell cultures. Following the discovery of a lymphokine activity in virus-infected kidney cell cultures, it was suggested that these various soluble substances represented a broad class of mediators of host defense secreted by cells and should more properly be called "cytokines."

Each cytokine binds to a specific cell-surface receptor. Subsequent cascades of intracellular signaling, mediated by (all of) a large group of enzymes, then alter cell functions. The alteration

may be up-regulation and/or down-regulation of several genes and their transcription factors, in turn resulting in the production of other cytokines, an increase in the number of surface receptors for other molecules, or the suppression of their own effect by feedback inhibition. Thus, cytokines are characterized by considerable "redundancy," in that many of them can share similar functions. In a comparable manner, cytokines are also pleiotropic, acting on different cells in the same way. The actions of cytokines may be grouped as autocrine, if the cytokine acts on the cell that secretes it, paracrine, if the action is restricted to cells in the immediate vicinity of a cytokine's secretion, and endocrine, if the cytokine is carried by blood or plasma to various regions of the body, where they can affect different tissues.

Cytokines are small, secreted proteins which mediate and regulate immunity, inflammation, and hematopoiesis. Most are produced *de novo* in response to an immune stimulus. They generally (although not always) act over short distances and short time spans and at very low concentrations and act by binding to specific membrane receptors, which then signal the cell via second messengers, often tyrosine kinases, to alter its behavior (gene expression). Responses to cytokines include increasing or decreasing expression of membrane proteins (including cytokine receptors), proliferation, and secretion of effector molecules. Most cytokines stimulate cell proliferation and differentiation but some cytokines are predominantly inhibitory. Cytokine activities are characterized using recombinant cytokines and purified cell populations *in vitro*, or with knockout mice with individual cytokine genes to characterize cytokine functions in vivo. Cytokines are made by many cell populations, but the predominant producers are helper T cells and monocytes (the precursors of macrophages and dendritic cells).

Keep in mind that the word "cytokine" is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukins (cytokines made by one type of leukocyte and acting on other leukocytes). These remarkable biochemicals can act synergistically or antagonistically. Their short half life, low plasma concentrations, pleiotropy, and redundancy all complicate the isolation and characterization of cytokines; therefore efforts to find new cytokines are made at the DNA level, by identifying genes similar to known cytokine genes.

The news, however, is not all good. A cytokine storm is a potentially fatal immune reaction consisting of a positive feedback loop between cytokines and immune cells, with resulting highly elevated levels of various cytokines. The cytokine storm is a failure of immune regulation, resulting in the release of more than 150 inflammatory mediators. Cytokine storms can (but do not always) occur in a number of infectious and non-infectious diseases including graft vs host disease, adult respiratory distress syndrome, sepsis, avian influenza, smallpox, and systemic inflammatory response syndrome. It is believed that cytokine storms were responsible for many of the deaths during the 1918 influenza pandemic, which killed a disproportionate number of young adults. In such cases, it is likely the virus either inappropriately activated effector cells and/or suppressed regulatory cells (or, likely, a combination of both) to create fatal disease. Preliminary research results from Hong Kong also indicated cytokine storms as the probable reason of many deaths during the SARS epidemic in 2003. Human deaths from the avian influenzavirus A H5N1 usually involve cytokine storms; elevated cytokine levels also have been detected in patients with fatal hantavirus pulmonary syndrome.

Keep in mind that there are three main aspects of immunity – innate, acquired, and regulatory. A cytokine storm appears to be a failure of regulation. The

function of T cells, cytokines, chemokines, and other bioactive chemicals is to either suppress or to activate, to kill the right things and to not kill the wrong things. Let's just say that a cytokine storm is a failure of regulation, an inappropriate response, and one that is likely to cause death, and let it go at that.

Other groups of cytokines include interferons and chemokines. The latter attract leukocytes to infection sites. Chemokines have conserved cysteine residues that allow them to be assigned to four groups (but enough detail is enough, so I will not outline their functions or characteristics).

Chemokines are a family of structurally-related glycoproteins with potent leukocyte activation or chemotactic activity. They are 70-90 amino acids in length and approximately 8 to 10 kDa in molecular weight. Most of them fit into two subfamilies with four cysteine residues. Establishment of these subfamilies are based on whether the two amino terminal cysteine residues are immediately adjacent or separated by one amino acid (immunologists are very "fussy"). The alpha chemokines, also known as CXC chemokines, contain a single amino acid between the first and second cysteine residues; the beta chemokines, or CC, chemokines have adjacent cysteine residues. Most CXC chemokines are chemoattractants for neutrophils, whereas CC chemokines generally attract monocytes, lymphocytes, basophils, and eosinophils. There are two other small subgroups but, thankfully, I do not have enough space here to describe them.

This non-review would be incomplete if it did not include at least a brief discussion of Toll-like receptors (TLRs). These comprise a class of non-catalytic pattern recognition receptors that recognize structurally conserved molecules derived from microbes that have breached the skin or intestinal mucosa, and which activate immune cell responses; they likely play a key role in the innate immune system. TLRs recognize molecules that are broadly shared by pathogens but distinguishable from host molecules. TLRs are present in many vertebrates, including mammals, as well as in invertebrates. Indeed, the original Toll protein was discovered in fruit flies (*Drosophila* spp) and, because TLRs have been found so widely in nature, they are thought to be ancient developmental proteins, also serving as immune defense mechanisms, established early in the evolutionary process. Toll-like receptors are now considered key molecules that alert the immune system to the presence of microbial infections. Thirteen TLRs have been recognized in humans. These are named TLR-1 to TLR-13, but you can be sure that these will be changed as soon as you learn them.

Following activation by microbial ligands, immune cells might produce cytokines, which activate signaling factors and initiate the inflammatory process. If it contains a bacterial ligand, the pathogen might be phagocytosed and digested, and its antigens presented to CD4+ T cells. If it contains a viral ligand, the infected cell may down-regulate its protein synthesis and undergo apoptosis. Immune cells that have detected a virus also may release anti-viral factors, such as interferon. When I naïvely asked them for a list of all known cytokines and chemokines, immunologist colleagues either gave me partial or outdated lists, kindly provided me with textbook chapters, or gave me e-mail addresses and Web sites where I might obtain such lists from commercial sources (there are many of these and they sell as many as 350 cluster of differentiation [CD] antigens and monoclonal antibodies to them, as well as other useful reagents). I now surmise that the reason many hundreds of chemokines have not been found is they have not yet been looked for sufficiently. I wrote to some of these commercial sources and they generously sent me some very nice wall posters that list alternative names, molecular weights, cellular expression data, ligand or receptor associations, and functions of these remark-

able bioactive chemicals. These posters have been taped to all the vertical parts of my office; as soon as I have all this memorized I will take them down and be able to see out of the windows again. One thing now is certain to me: an immunologist must be able to draw arrows and dotted lines.

In sum, what these people are talking about is not yet clear to me; immunology-speak still is like a foreign language. Foreign languages are not foreign to those speaking them, so I give immunologists the benefit of the doubt and assume they know what they are talking about, much as I do with mathematicians and astronomers. Immunology will simply have to remain another area of my ignorance. I apologize for not being helpful to the reader but I take no blame for the problem. This is a complex area and, it seems, nothing will make it simple enough for me.

Acknowledgments:

I thank Drs Tian Wang, Colorado State University, Fort Collins, Colorado and Tony Schountz, University of Northern Colorado, Greeley, Colorado, each a skilled immunologist, for their assistance in finding the wall posters that clutter my office and for trying to teach me something. I am not their fault. I also am grateful to Dr David White, Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, for trying to clarify the unclarifiable (for me); I would not be surprised if he denies participating in this effort, not to speak of admitting that I plagiarized a few of his sentences and thoughts.

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