6/30/87

Perry A. Chapdelaine, Sr.
The Rheumatoid Disease Foundation
Rt. 4, Box 137
Franklin, TN 37064

Dear Perry:

Enclosed is a copy of my recent progress report to Paul Pybus. I figure that we have enough funds to sustain the inertia for two months. I know you are doing all that you can. Best wishes.

Sincerely yours,

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6/28/87

Dr. Paul K. Pybus
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Dear Dr. Pybus:

I was surprised to hear that my letter to you of 2/17/87 took so long to arrive. Your correspondence of 5/21/87 arrived the first week of June. I don't know what was the problem, especially since my prior correspondence to you apparently had arrived within a reasonably short time after being mailed. Furthermore, a copy of the 5/21 letter to you that I sent to Perry Chapdelaine arrived promptly. I will have a talk with the head of the post office here to insure the expeditious handling of this and all future correspondence.

First, I will try to answer some of the questions asked in your letter (others will be answered below in the body of this report). "Lymphokines" are the soluble mediators of a number of immunologic functions. I discussed the ones thought to be most relevant to rheumatoid arthritis in my report of 11/6/86. In that report, however, I used the term "cytokines." Depending on the cell type releasing the mediator, they are called "lymphokines" (i.e., from lymphocytes), "monokines" (i.e., from monocytes/macrophages), etc. "Cytokines" is a general term for these hormone-like molecules that encompasses lymphokines, monokines, and other factors produced and released by tissues which primarily exert their effect within a local site (paracrine). I tend to use the terms "lymphokine" and "cytokine" interchangeably. Perhaps that was the reason for the confusion.

Lymphokines and monokines are produced as a result from the interactions of lymphocytes and monocytes. They influence the inflammatory process in a variety of ways, including the infiltration of leukocytes into the area (chemotactic factors), the state of activation of the inflammatory cells within the site of inflammation, and direct effects on the surrounding tissue. Well over one hundred lymphokines and monokines have been described, based on their biologic activity. The actual number is probably considerably less, in that the same lymphokine or monokine may affect several cellular functions, several different cell types, or the same cell differently depending on its stage of maturation.
For example, interleukin 1 (IL-1) (described below), has been called "lymphocyte activating factor," "osteoclast activating factor," "endogenous pyrogen," "proteolysis inducing factor," "catabolin," and "serum amyloid A inducer," among other names, depending on the biologic activity under observation. The gene for IL-1 has been cloned, and it has thus been proved that the same molecule can exert these many and varied biological effects on different cells and tissues.

Regarding your question on the meaning of the "lymphocyte-to-target cell ratio": Cytotoxic T lymphocytes (CTL) recognized antigens on the surfaces of host cells (e.g., viruses, alter host proteins, etc.), and attempt to eliminate the antigen by lysing the cell bearing the stimulating antigen. Thus, the "target cell" was a cell bearing the same antigen as was used to originally induce the CTL. For the purpose of the assay, the antigenic "target" cell is radiolabeled with an isotope, and release of the isotope into the supernatant is measured as an index of CTL activity.

You are correct in your perception that not much is known regarding the immunoregulatory effects of the nonsteroidal antiinflammatory drugs. Much is known pharmacologically and biochemically about how these drugs relieve symptoms of inflammation, but seeing as immune stimulation is what causes the inflammation to become chronic, specific effects on the immune response warrant detailed investigation. Since knowledge of the regulation of the immune system by lymphokines and monokines is recent, a limited range of drugs have been tested.

Now to describe our recent laboratory studies and findings:

Initiation of T cell responses requires the participation of macrophages that express "Ia" ("Immune associated") molecules on their surface - proteins coded for by genes in the I-region ("Immune") of the human major histocompatibility complex on chromosome 6. Ia molecules are required in order for the macrophage to have the capacity to function as an antigen-presenting cells to the T cell. Incubation of macrophages with lymphokines resulted in an increased level of Ia expression. This increase was not inhibited by clotrimazole or metronidazole.

As a result of the T cell-macrophage interaction, macrophages produce the monokine, IL-1. It is of considerable interest in rheumatoid arthritis because it affects numerous cell types, including: chondrocytes and fibroblasts (production of prostaglandins, collagenase, plasminogen activator from cartilage); neutrophils (metabolic activation, chemotaxis); synovial cells (proliferate, prostaglandins, collagenase); hepatocytes
(acute phase reactant proteins); osteoclasts (collagenase, bone resorption); brain (prostaglandins, fever, somnolence, anorexia). When we looked to see if clotrimazole or metronidazole inhibited macrophage IL-1 production, we observed no effect.

Activation of macrophages and polymorphonuclear neutrophils with complement components, immune complexes, lymphokines, etc. stimulates an increase in oxygen production – the so-called "respiratory burst." The oxygen is used to form oxygen free radicals (e.g., superoxide anion and hydroxyl radical). These extremely reactive products (they have an extra pair of electrons in their outer orbital) are normally used to kill intracellular pathogens that have been phagocytized. In rheumatoid arthritis, however, they are thought to leak out and cause tissue destruction. Inhibition of oxygen free radical formation may be the mechanism behind superoxide dismutase (SOD) therapy, which breaks down superoxide anion, and a mode of action of copper, which can have SOD-like activity in metal complexes.

It does appear as though clotrimazole, but not metronidazole, is capable of inhibiting superoxide anion formation by neutrophils, although at concentrations that may not be pharmacological. We are in the process of standardizing a more sensitive assay for superoxide anion, in order to better detect effects of clotrimazole at lower concentrations.

You are correct in your point that the degree of activation of the macrophage may alter the apparent effectiveness of drugs. Certain macrophage functions may be drug sensitive at one stage in their development but not another. We are aware of this possibility, and in our studies we look for drug effects on macrophages in different states of activation – normal "resident" macrophages, macrophages elicited by injection of a sterile irritant (Brewer’s thioglycollate broth), and macrophages immunologically activated by injection of a vaccine of Corynebacterium parvum.

Other aspects of macrophage function are being investigated. Macrophages produce a range of products that could contribute to the inflammation, e.g., plasminogen activator, collagenase, elastase, proteoglycanases, acid hydrolases, phospholipases, etc. Furthermore, in rheumatoid arthritis there are reports that macrophage-derived factors can elicit the surrounding tissue cells to contribute to the inflammation by producing collagenase, plasminogen activator, collagen, prostaglandins, etc. Thus, clotrimazole may affect the tissue reaction to inflammatory substances. Many cellular sites of attack by the drug remain to be investigated.
I would say at this point in our assessment of clotrimazole that our best working hypothesis remains that the mode of action is due to inhibition of T lymphocyte function and/or inhibition of phospholipase A\textsubscript{2} (PLA\textsubscript{2}). Whether clotrimazole has a complex mechanism of action and these two sets of observations represent different effects on cellular functions, or whether a link may exist between the particular effects of clotrimazole on T cells and anti-PLA\textsubscript{2} activity, is an area in which Dr. Franson and I are closely collaborating. We have some evidence that T cell activation and PLA\textsubscript{2} are coupled.

As more effects of clotrimazole are observed, we hope that a pattern will emerge which will allow us to associate the drug’s effect on specific components of the inflammatory response with the clinical observations. Although it is important to dissect \textit{in vitro} the potential therapeutic effects of the drug on various components of inflammation, confirmatory evidence can only be gathered by pathophysiologic studies in experimental animals. Parallel studies conducted \textit{in vitro} and \textit{in vivo} will be required in order to generate definitive data on the therapeutic mode of action of clotrimazole. Therefore, we have recently initiated a series of studies using an animal model of rheumatoid arthritis.

In rats, synovitis similar to that of human rheumatoid arthritis can be induced by injecting complete Freund’s adjuvant (mineral oil containing cell protein from mycobacteria). Rat adjuvant arthritis is one of the most useful models for evaluation of drugs in the treatment of rheumatoid arthritis. Immunopathologic events cause the disease, with the T cell playing the central role. (Indeed, the arthritis can be transferred to normal, naive animals just by adoptive transfer of T cells from arthritic animals). Of course, no model is a faithful representative of human disease. Sufficient mechanistic similarities exist, however, for it to have a predictive value.

This brings you up-to-date on where our efforts are focused and the avenues along which I am thinking. In summary, the project is progressing on several fronts which we feel have the potential to pinpoint theoretical mechanisms for the therapeutic effectiveness of the drug. Inferences gathered \textit{in vitro} regarding the immunoregulatory characteristics of clotrimazole will soon be tested on the disease condition \textit{in vivo}. 
As for our source of clotrimazole, we purchase it through the Sigma Chemical Company, St. Louis, Missouri, USA. The product which they sell, however, is not licensed for use in humans, although biochemically it is the same as Bayer's.

I hope that you are well. Thank you for the encouraging remarks in your recent letter. Till I hear from you again, I remain,

Sincerely yours,

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cc: Mr. Perry Chapdelaine, Sr.