Medical College of Virginia
Virginia Commonwealth University

March 8, 1985

Perry A. Chapdelaine, Sr.
Executive Director/Secretary
Rheumatoid Disease Foundation
Rt. 4, Box 137
Franklin, TN 37064

Dear Mr. Chapdelaine:

I have received your letter of February 14, 1985 and am gratified by your favorable remarks. Excuse me for not having replied sooner; this is a very busy time of the year for me. I am glad to take this opportunity, however, to respond to your letter and the questions that were raised.

Regarding the effects of centrifugation and low temperature: Thank you for passing along the information that centrifugation is not deleterious if carried out at a low speed. This is what one would suppose. Using buoyant density centrifugation may be more gentle still on the cells, and also remove contaminating tissue debris that would otherwise sediment with the amebae. I am surprised to hear that chilling may have a harmful effect. Cold usually helps to counter the pernicious effects inherent in most procedures that dissociate pathogens from tissue. We will be mindful of both these factors in our isolation attempts.

Regarding Koch's postulates and genetic susceptibility: Although Koch's postulates have proven invaluable in identifying pathogens, they cannot always be met. Reasons sometimes involve genetic susceptibility. This is not a factor in the proposed research because to establish rheumatoid disease in experimental animals is not among the present specific aims. This would be an objective in future investigations, however. Therefore going back to the point which you raised, association between rheumatoid disease and the human major histocompatibility complex has been established, specifically involving the HLA-DR4 gene marker. HLA DR genes are associated with susceptibility to a variety of diseases, by virtue of the fact that they regulate immunologic responses. Analogous immune response genes have been established for a number of species, including most notably mice, rats, and guinea pigs. Since rheumatoid disease in man is associated with immune response genes and immune response genes have been identified for other species as well, and since Dr. Wyburn-Mason reported isolation of the limax amebae from animals, there is a good probability that we will be able to establish an animal model when the time comes.

Regarding whether adaptation of the ameba to in vitro culture will alter its characteristics: A general pronouncement of this question is a caveat of the in vitro approach for the study of any pathogen, and one must take heed. In the context of my proposal it is of concern primarily for the serological aspects. Most pathogens in vitro do retain an antigenic profile similar to their in vivo counterparts. As a precaution, however, we will screen antisera and monoclonal antibodies raised against culture forms of the amebae against fresh isolates.

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To answer two additional technical questions you raised, which pertain to studies outside of the scope of the present proposal (though we would be capable of developing them after performing the preliminary studies put forth in the application): 1) For the purpose of drug screening, one would want to use amebae from cultures with growth-arrested bacteria (heat killed or antibiotic-inhibited) or from axenic cultures so that conditions during the test period are not influenced by effects of or on the associate organism. 2) I am of the opinion that a broad panel of serologic reagents could be developed against cell surface markers on different species of limax amebae that would be useful for taxonomic purposes.

In publishing the results from the investigation, support from the Rheumatoid Disease Foundation will be acknowledged. Furthermore, I am agreeable to the Foundation's utilization of the results for publicity or fund raising purposes, with the precondition of my approval of the accurate presentation of the findings.

(804) 786-0348

Regarding patent rights: I am not qualified to comment on behalf of the University. The official who handles such matters is Mr. Herbert Chermside, Director of Sponsored Programs Administration. You can address your questions regarding patent rights, licensing, sharing of royalties, etc. to him at Box 568, Medical College of Virginia, Richmond, VA 23298.

Finally, you raise the relevant and appropriate question of what if, despite our diligent efforts, attempts to isolate the ameba are unsuccessful. For verification of the Wyburn-Mason hypothesis isolation of the putative pathogen is crucial. This is the explicit reason for choosing the specific aims advocated in the proposal, even while recognizing the potential impasse. There is room, however, for lateral thinking. The cooperative interactions between pathologists, rheumatologists, and basic scientists involved in initiating this project will automatically result in the generation of new ideas and approaches. Therefore if we run into a predicament, after consultation with my collaborators I would channel the course of the investigation along new lines where it is felt progress can be made. An alternative area of investigation consistent with the accumulated expertise of the group, for example, would be an examination of the effects of the anti-amebic medications used as part of the Foundation's recommended therapy on the subsets of cells (T cells, B cells, macrophages, etc.) mediating and regulating the inflammatory reaction of the synovium in rheumatoid disease. At the time of such an eventuality we would specify our new research initiatives for the Foundation's directorate. Overall, I feel confident that we will be able to make progress in a direction consistent with objectives of the Foundation, and funds granted by the Foundation will be expended consonantly.

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I trust that my responses to these questions will be helpful in supporting my application. If there are any additional questions, do not hesitate to write.

Sincerely yours,

Brian M. Susskind

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