

Cytolytic T Cells to Melanocytes in Vitiligo

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The grant awarded in 1995 to support our research in vitiligo, was used to conduct 2 projects. The first was to begin studies to determine the abnormal immune responses to melanocytes present in patients with vitiligo also involve lymphocytes. These are circulating blood cells which have the capacity to selectively attack and destroy certain tissues. Lymphocytes play the major role in causing certain autoimmune disease in destroying cancer cells, so it is reasonable to suspect that they might also be involved in the cause of vitiligo. The study of lymphocytes is much more difficult than that of antibodies, and for that reason, very little work has been done on studying their involvement in vitiligo. With recent support of the NVFI, we have developed a novel assay to study lymphocyte responses to melanocytes. This assay is much more sensitive and quantitative than prior assays. We have demonstrated the effectiveness of the assay in patients with melanoma, where a lymphocyte response against malignant pigment cells is known to be present. Having now demonstrated that the assay works, we are beginning to apply it to patients with vitiligo. Studies have just begun and the results are not yet available.

The second was to determine whether the autoantibodies to pigment cells which are present in vitiligo are directed to tyrosinase. The results of this work are summarized in an abstract which has been submitted for presentation to the forthcoming annual meeting to the *Society for Investigative Dermatology*. Vitiligo is associated with antibodies to melanocyte antigens. Some of these are similar in size to tyrosinase, suggesting that melanocytes antibodies in vitiligo are directed in part to tyrosinase. To test this hypothesis, sera of 40 patients with active vitiligo and 60 control individuals without vitiligo were tested for antibodies to tyrosinase in extract of cultured human melanocytes by immunoblotting, immunoprecipitation and sandwich " ELISA. By immunoblotting, there was no difference between vitiligo and control sera in the incidence of antibodies to antigens that co migrated with tyrosinase identified by the DOPA reaction and with a specific antibody. By immunoprecipitation, sera of 20 patients with active vitiligo and 20 control individuals were reacted with the melanocyte extract, bound proteins precipitated with protein- A sepharose, run on SDS-PAGE, and tested for tyrosinase by DOPA reaction. Although DOPA reactive tyrosinase was precipitated by the control anti-tyrosinase antibody, it was not by any vitiligo sera. Finally, anti-tyrosinase antibody was used in a sandwich-ELISA assay to test 13 vitiligo and 7 control sera for antibodies to tyrosinase. Again, there was no significant difference in average tyrosinase binding activity between vitiligo and control sera. Thus, vitiligo does not appear to be associated with antibodies to tyrosinase.