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Investigation of Immunoregulatory Alphaglobulin (IRA) in Shock and Trauma

Annual Progress Report

John A. Mannick, M.D.

October 13, 1977

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No. DAMD 17-76-C-6076

Peter Bent Brigham Hospital
Boston, Massachusetts 02115

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The major accomplishment of the past year of research supported by Contract number DAMD-17-76-C-6076 was the investigation of the nature of the immunosuppressive material present in the serum of patients sustaining major trauma or burns. ~~We have~~ fractionated ~~the~~ serum of such patients showed ~~and have found~~ the immunosuppressive activity resides in a low molecular weight polypeptide fraction which in turn has been fractionated by sephadex chromatography and high voltage electrophoresis. The active moeity appears

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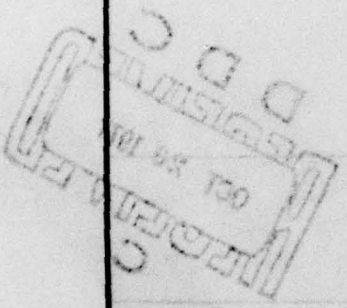
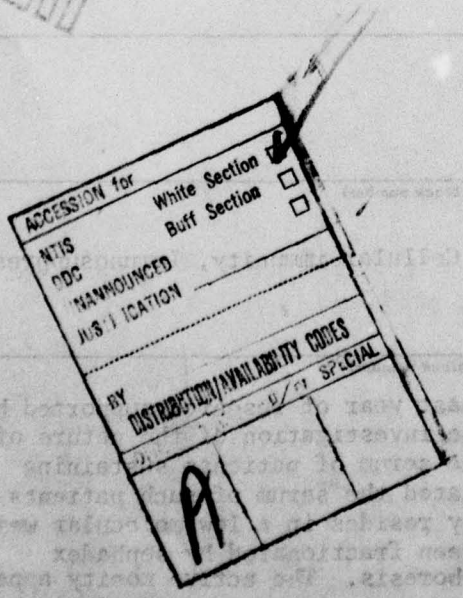
to be a basic peptide resolved by high voltage electrophoresis. During the past year we have simultaneously studied the response of lymphocytes recovered from the peripheral blood of patients sustaining major trauma and burns and have noted that the lymphocytes of many such patients are deficient in their ability to respond to mitogen stimulation in vitro and in their ability to form rosettes with sheep red blood cells. An attempt is currently being made to determine the correlation between hyporesponsiveness of lymphocytes from traumatized and burn patients and the suppressive activity of the serum from these individuals as tested against normal human lymphocytes.





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PROGRESS REPORT - ANNUAL

For the past three years with the support of this contract we have recovered an immunosuppressive polypeptide in trace amounts from pooled normal human serum. This peptide fraction, which we have called immunoregulatory alphaglobulin (IRA), is probably carried non-covalently bound to an alphaglobulin carrier or carriers at normal pH. It is released from its loosely bound state under conditions of high ionic strength and acidic pH. The IRA peptide has a molecular weight of 1000-2000 and chemically appears to be entirely a polypeptide by the quantitative Biuret test. It contains no nucleic acids, neutral sugars, scialic acid or lipids. It also contains no cortisol, prostaglandins E_1 or E_2 or ribonuclease activity.

We have shown that IRA inhibits a wide variety of T-cell mediated immune responses including the rejection of skin allografts in mice, the rejection of renal allotransplants in rats, immunity to syngeneic tumors in mice, the production of MIF by specifically sensitized guinea pig T-cells, the proliferative response of human and animal lymphocytes in vitro to T-cell mitogens, such as phytohemagglutinin (PHA) and to specific antigens to which the lymphocyte donor is known to be sensitized. We have shown that IRA is non-toxic to lymphocytes or experimental animals. It appears to act by preventing the recognition of antigen by potentially responsive T-lymphocytes. It is ineffective if it is andministered after the antigen to be studied. Lymphocytes exposed to IRA will respond normally in tissue culture to antigenic and mitogenic stimuli if they are thoroughly washed and then recultured in medium lacking IRA. IRA has no effect on B-cell immune responses, however, it markedly inhibits antibody formation to antigens which require T-helper function, such as the plaque-forming cell response of mice to sheep erythrocytes (SRBC).

During the past two years we have studied a group of 117 patients who have been subjected to major trauma, including major surgery, or who have sustained burns. We have found that varying percentages of this group of patients have immunosuppressive serum, defined as serum which will inhibit by 50% or more the in vitro stimulation of normal human peripheral blood lymphocytes by phytohemagglutinin (PHA), when compared to normal human AB serum and/or autologous serum. The percentage of patients having immunosuppressive serum has been found to vary with the severity of the truama and with the number of complications sustained by the patient. Patients who have septic complications are particularly likely to have suppressive serum. The immunosuppressive activity in trauma patients' serum does not appear to be caused by anesthetic agents since patients undergoing general anesthesia for minor surgery do not develop suppressive serum.

We have fractionated the serum of many of these individuals by DEAE cellulose chromatography and have found that the majority of the immunosuppressive activity is recovered in Peak I while the immunosuppressive activity in normal patients' serum is recovered in later alphaglobulin rich peaks. We have also found that the immunosuppressive activity contained in Peak I from trauma patients can be recovered after acidification and diafiltration, as a peptide fraction of less than 2,000 molecular weight, which is very highly suppressive of T-cell function both in vivo and in vitro. It appears, therefore, that patients who have been recently traumatized have high levels of a circulating immunosuppressive peptide fraction which appears to be similar to IRA peptide. The peptide obtained has been investigated chemically to determine its true peptide content by the quantitative Biuret test and has been found to be entirely composed of polypeptide as far as can be determined by this assay. The presence of nucleic acids and carbohydrates have been ruled out by standard chemical techniques and no cortisol is present as determined by the competitive protein binding assay. Similarly, the peptide contains no prostaglandins E_1 and E_2 as determined by radioimmunoassay.

More recently we have attempted to fractionate the immunosuppressive peptide obtained from trauma serum by G25 sephadex gel filtration and finally by preparative high voltage electrophoresis. In initial studies it appears that a good share of the immunosuppressive activity in this peptide fraction is contained in the peptides at the basic end of the electrophoresis pattern. The active peptide in normal serum and in serum from cancer patients also appears to be located in the same area by preparative high voltage electrophoresis. We have recently been exploring a technique to fractionate the serum from trauma patients and cancer patients by high pressure liquid chromatography in an attempt to purify the suppressive peptide moeity. Attempts at amino acid analysis of the basic polypeptide obtained by high voltage electrophoresis have been impaired by the fact that the peptide must be eluted from paper and, therefore, the final product is contaminated by amino acids from the paper. The advantage of high pressure liquid chromatography, if the peptide can be isolated by this method, is that the product will be free of salt or amino acid contamination. The trauma serum peptide when isolated will be subjected to amino acid analysis and ultimately to amino acid sequencing. Its amino acid composition will then be compared with that of similar peptides obtained from cancer serum and normal serum.

During the past year we have continued a study of the lymphocytes of trauma patients with regard to their response to PHA stimulation in vitro and their ability to form rosettes with sheep red blood cells (SRBC). During the past 6 months 20 patients have been studied. As anticipated the lymphocytes of patients who have suffered major burns or the most severe trauma are suppressed with respect to their ability

to respond to PHA stimulation and with regard to their ability to form rosettes with SRBC (E-rosettes) as compared with control lymphocytes from normal individuals, even when incubated in the same normal AB reference serum. In some trauma patients whose lymphocyte responsiveness in vitro is impaired, the response has returned to or toward normal after washing the lymphocytes 6 times as opposed to once in vitro in tissue culture medium prior to culture with PHA or exposure to SRBC. There is also a correlation between the hyporesponsiveness of lymphocytes from traumatized and burn patients and the suppressive activity of the serum from these individuals as tested against normal lymphocytes. However, this correlation is only rough at present and more patients must be studied. It appears that the more suppressive the serum the more likely it is that the patient's lymphocytes are hyporesponsive in normal serum after one washing in vitro. Thus it appears that one reason for the hyporesponsiveness of the lymphocytes of trauma patients as reported by a number of workers is likely to be lymphocyte surface coating with a substance which can be removed by multiple washings in tissue culture medium. We are currently studying the medium in which the lymphocytes have been washed in an attempt to obtain the suppressive material from this source.

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