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TECHNICAL REPORT NO. 1

IMMUNOREGULATION TO FACILITATE TRANSPLANTATION AND REPARATIVE SURGERY: DEVELOPMENT OF NATURAL BIOLOGICAL AGENTS.

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CONTENTS

CONTENTS (Continued)

Immunoregulation to Facilitate Transplantation and Reparative Surgery: Development of Natural Biological Agents

A. INTRODUCTION

The following technical report summarizes the first year's progress on ONR Contract N000-14-77-C-0748 "Immunoregulation to Facilitate Transplantation and Reparative Surgery: Development of Natural Biological Agents", which was initiated September 15, 1977 for the purpose of developing a system for prolonging graft survival, without compromising the general health of a transplant recipient.

B. BACKGROUND

Limb injuries are among the most common of all battle casualties and although modern methods of rapid evacuation, treatment, and reparative surgery have greatly improved the chances of survival for victims of such casualties, full recovery of limb function is not possible in many cases. The development of a program whereby permanently injured limbs could be replaced by a fully functional transplant from a cadaveric donor would permit, in many instances, complete recovery from battle casualties or at least reduced disability from this form of injury.

Although there still remains a large number of surgical and neurological problems to be solved before therapeutic application of limb transplantation becomes a reality, the

majority of these are technical and should be solved as more precise surgical procedures are developed. Currently, the most formidable barrier to the successful application of limb transplantation is the normal ability of the host to recognize and destroy foreign tissues in the process of graft rejection. Thus even the most exquisite methods of limb transplantation will be useless unless the normal immune system can somehow be modulated to accept the graft. Furthermore, since most candidates are healthy individuals, and the transplant is not a life sustaining requirement, current immunosuppressive measures such as irradiation, or drug treatment, which prolong organ graft survival, cannot be used, since they offer a greater threat to the individual's life than the absence of the limb.

Over the past 30 years it has become clear that circulating lymphocytes are capable of responding to foreign tissue antigens. These lymphocytes fall into two main categories, those derived from the thymus gland (T cells) and those derived from the bone marrow (B cells). It is now known that both types of cells collaborate together for the production of antibody, and while the B cells actually produce the antibody, the T cells in some way regulate this event. The T cells can further be functionally divided into helper cells - those that help B cells to produce antibody, suppressor cells - those that suppress the response of helper cells, and killer cells - those that are capable of killing foreign tissue cells.

It is now known that surface receptors on both T and B cells interact with antigen (foreign proteins) in a similar manner, suggesting that structurally these receptors are in some ways related. This is shown schematically in Figure 1. The site where the receptor folds around or "binds" the antigen has been called the combining site or idiotype region. All antibody molecules derived from a clone of B cells are specific for a particular antigen and have the same combining site (idiotype). Also, the receptors on both T and B cells, express the same idiotypes.

It is now possible in a limited way to produce anti-idiotype antibodies to various lymphocyte surface receptors. Thus, the use of these anti-idiotype antibodies, is a potentially powerful tool for detecting and inactivating cells bearing receptors of a given specificity. It has been the aim of this contract to prepare anti-idiotype antibodies against the surface receptors for transplantation antigens thereby blocking or eliminating those clones of cells reactive to that particular transplantation antigen, allowing the transplant to be performed without the usual rejection mechanisms in action. An added benefit is that other cells, which must deal with infectious agents, are not in any way inactivated.

It has recently been suggested that anti-idiotype antibodies are the basis of a normal feedback control system whereby antibody responses are eventually damped, rather than

allowed to go on indefinitely (Network Theory). Hence we are exploring what is thought to be a natural immunoregulatory system.

It is also known that once the antigen and antibody have combined to form an immune complex, these complexes are capable of modifying the immune response in both a positive and negative fashion. Although the mechanisms underlying this type of modification are currently unclear, they offer the possibility of utilizing another type of natural regulator for specific modulation of the immune response.

C. RESEARCH DESIGN AND PLANS

The initial phases of this contract were to follow a sequence of events:

(1) Establishment of a contract facility research laboratory with capabilities of studying *in vitro* immune responses and methods of manipulation of these responses.

(2) Preparation of antiidiotype antisera for various protein antigens.

(3) Characterization of the immunoregulatory activities of the antiidiotype antibody for these protein antigens and analysis of the shared idiotypic specificities among T and B lymphocytes.

(4) Preparation of soluble immune complexes and assay of the effect of these immune complexes on various aspects of the immune system and including the interactions among the various lymphocyte subpopulations.

(5) Evaluation of the combined activity of antiidiotype and immune complexes on immune responses.

Depending on the results similar studies could then be initiated in primates, to evaluate the clinical relevance of these reagents.

D. RESULTS

1. Preparation of antiidiotype antisera.

The protocol for production of anti-idiotype antibody against a cell surface receptor, is simply to generate cells responsive to a given antigen in vitro, enrich for these cells, and inject them back into a syngeneic host or F_1 animal. In this way the animal should produce antibodies against the specific receptor.

1.1 Scientific Basis. The rationale for this approach is based on certain predictions from the Clonal Selection Theory, proposed by Burnet in 1959, and the Network Theory proposed by Jerne in 1974.

The clonal selection theory proposes that during the development of the immune system, those cells bearing specificities for autologous (self) antigens are inactivated or destroyed by contact with the relevant self antigen (clonal abortion). However, this phenomenon is considered to be dose dependent rather than absolute. In the normal animal, the number of cells capable of responding to any single antigen

is very small, and therefore the representation of any single antigen receptor is also very small. The injection of large numbers of cells bearing surface components identical to those of the host except for the unique antigen specific receptor results in the selective recognition of this receptor as being "foreign", as it has never been experienced in this vast quantity. The host then makes antibodies against its own receptor and in essence forms an autoanti-idiotype antibody.

The Network Theory proposed that, the production of antiidiotype antibodies is a normal feedback mechanism by which the immune response is damped and finally halted. Recent work has shown that this is the case for responses to certain antigens. We are proposing to prepare antiidiotype antibody by the injection of large numbers of antigen-specific cells into syngeneic hosts. The antibody once prepared will be tested and used to modulate the immune response to the specific antigens.

1.2 Facilities Requirement. Since the preparation of antiidiotype antisera is a long-term project requiring multiple immunizations of animals, our first objective was to establish an animal facility where the animals could be housed free of disease for long periods. To accomplish this we are now housing our animals in horizontal flow laminar flow hoods, which maintains the animals free of airborne contaminants, thereby decreasing the chance of endemic infection within the group of mice we are utilizing.

1.3 Model System. As a model system we have chosen to evaluate the in vitro responses to KLH (Keyhole Limpet Hemocyanin) which is an extremely powerful antigen. The project plan calls for the immunization of cells in vitro with KLH, followed by enrichment of the T cells that respond to KLH using various parameters such as the size of the cells, their non-adherence to nylon wool, and lack of susceptibility to freezing.

a. Generation of antigen specific helper cells.

The ability to generate antigen specific helper cells to KLH in vitro is shown in Figure 2. In this assay system spleen cells from normal mice are incubated with the antigen TNP-KLH and syngeneic KLH primed helper cells. The T Cells have been previously primed in vitro to KLH, and are added on a graded basis. The figure shows that as more KLH helper cells are added, the response of the normal spleen cells increases up until no further effect is seen. The decrease in response seen at 10^5 and 10^6 is thought to represent suppressor cell activity at the higher number of cells. Other experiments have shown that the degree of help is variable from experiment to experiment, and that the optimum number of helper cells ranges from 10^4 to 10^6.

b. Enrichment of antigen specific helper cells.

Next we chose to study methods of enriching for the antigen specific helper cells. The first was passage of in vitro primed helper cells through nylon wool columns, the

results of this study are seen in Figure 3. It should be noted that passage through nylon wool, removes B cells, macrophages, and other adherent cells, leaving a more active T lymphocyte population. While this experiment demonstrates only slight purification, others suggest a one order of magnitude enrichment using nylon wool.

c. Effect of freezing on helper cell function

Since the immunization of many animals will require storage of large numbers of cells, it was relevant to learn if the primed helper cells could be stored frozen, then thawed and subsequently utilized. We studied this question by preparing helper immunizing cells to KLH in vitro, freezing them in a controlled rate freezer, storing them for approximately one week in liquid nitrogen, thawing them out, and utilizing them in our standard in vitro assay. The results of this particular study can be seen in Figure 4. It is clear that cells either fresh or frozen are capable of providing the help necessary for B cells to go on to make antibody; in fact it appears that frozen cells might be somewhat superior, however additional experiments are needed to confirm this.

These studies taken together then suggest that our in vitro assay is functioning and that we are able to generate, and enrich antigen specific KLH helper cells. While it is not clear how many of the end product cells are actually

responding to KLH, it is estimated that approximately 5-10 out of 100 of the cells are KLH reactive, however it may be that the frequency is as low as 1 in 1000 or 10,000. Assays are being planned that will address this question.

d. Application of macro culture techniques.

Since it was our intent to immunize large numbers of animals with these purified KLH helper cells, techniques were undertaken to scale up the in vitro generation of helper cells to meet the enormous cellular requirement. Table 1 summarizes the results of studies utilizing extremely large culture vessels to prepare helper cells. It would appear that 600 x 10^6 cells/culture seems to be an optimum for generating active helper cells in these tissue culture flasks.

e. Immunization of animals.

With the establishment of methods whereby large numbers of helper cells could be prepared, and enriched to a limited degree, primary immunization of animals for the preparation of antiidiotype antisera have been completed. It is anticipated that 10-20 immunizations of these animals at weekly intervals may be necessary to stimulate production of anti-idiotype antibody.

f. Development of new enrichment techniques, studies using soft agar cloning.

While these initial attempts at preparing and enriching antigen specific helper cells have been successful, it is our

aim to attempt to prepare cells purified on a much higher scale, hopefully to such a degree that only cells reacting to KLH could be injected. This would no doubt enhance the immunogenecity of these cells and produce a far better anti-idiotype antibody. Methods under evaluation include enrichment of helper cells on density gradients to select for blast cells, and cloning the enriched cells in soft agar, with large scale screening for helper cell activity. This later method would allow us to grow specific helper cells in tissue culture, in vast quantities.

2. Studies on antigen antibody complexes. Initial work on the generation of antigen antibody complexes has dealt mainly with the production of sufficient amounts of highly specific antibody in order to prepare the immune complexes. It is anticipated that these reagents will be available in the near future and studies concerning the selection of appropriate antigen-antibody ratios will be undertaken.

3. Conclusions from research completed. As expected, the vast majority of the initial years work has been largely technical in that the experimental system was implemented for the first time in our laboratory. The results show that large numbers of cells reacting to complex antigens can be generated in vitro, stored, and recovered essentially intact for use at a later time. Although this aspect of the work is purely technical from the viewpoint of our project, such

information may prove valuable to investigators interested in providing heightened immunity to certain types of infection, through passive administration of lymphocytes activated to antigens characteristic of the infectious agent.

Although we are quite satified with the status of this project after one year, there is one technical aspect which is not yet optimal. This is the great variation in the level of response generated in the Marbrook culture system, and we hope to improve this aspect of the project in the next year.

4. Proposal for continuation of this contract

The initial success and high yield of important data on this contract has provided encouragement that continued pursuit of these efforts would be beneficial. We would propose to continue the workscope essentially as outlined, continuing our studies on the roles of these biologically active agents in immunoregulation. Data from these sorts of studies will be made available to individuals at the Naval Medical Research Institute as well as to those funded under other ONR contracts dealing with the development and expanded use of various immunosuppressive agents.

5. Publications. One publication entitled "Human Antigen Specific Helper T Cell Factors and B Cell Responses" was accepted for publication in "In Vitro Induction and Measurements of Antibody Synthesis in Man", edited by A.S. Fauci and R.E. Ballieux, Academic Press, 1978 (In Press). A copy of this manuscript is attached.

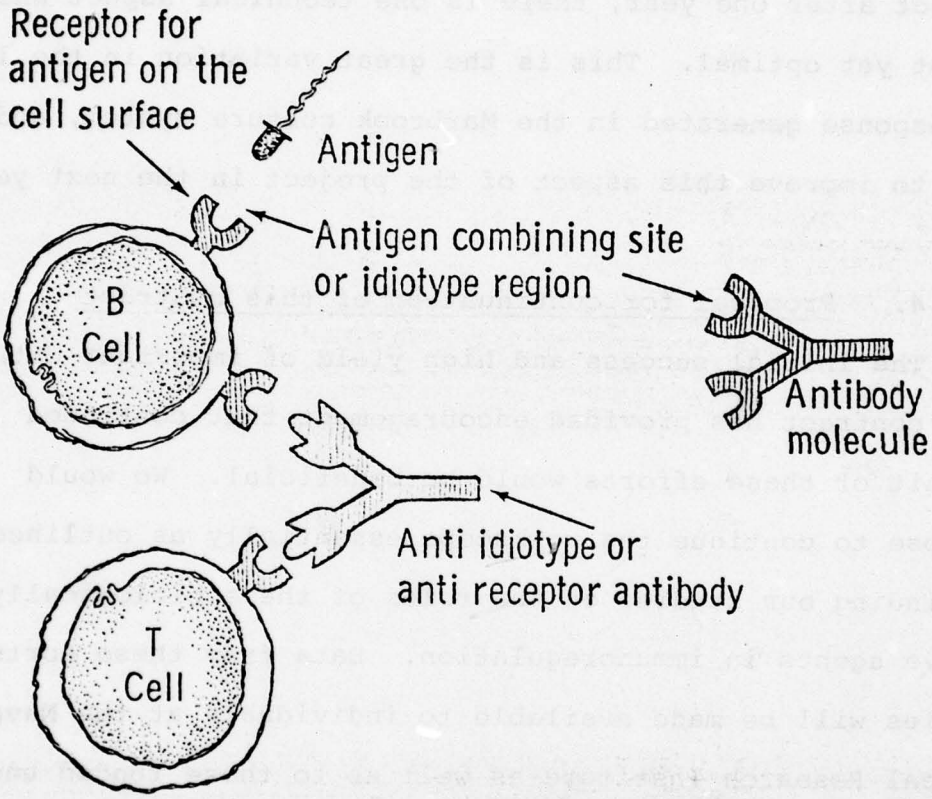

FIGURE 1

SCHEMATIC DIAGRAM OF CELL SURFACE RECEPTORS,
COMBINING SITES, ANTIBODIES, AND ANTI IDIOTYPE ANTIBODIES

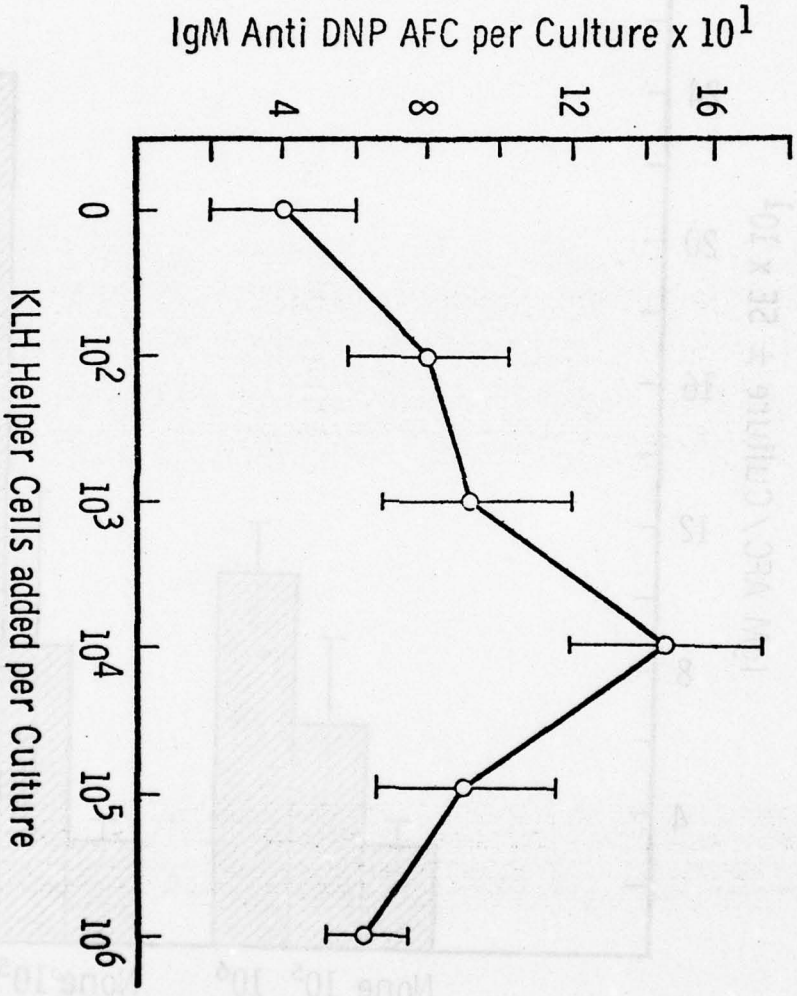

FIGURE 2. ABILITY TO GENERATE KLH HELPER CELLS IN VITRO

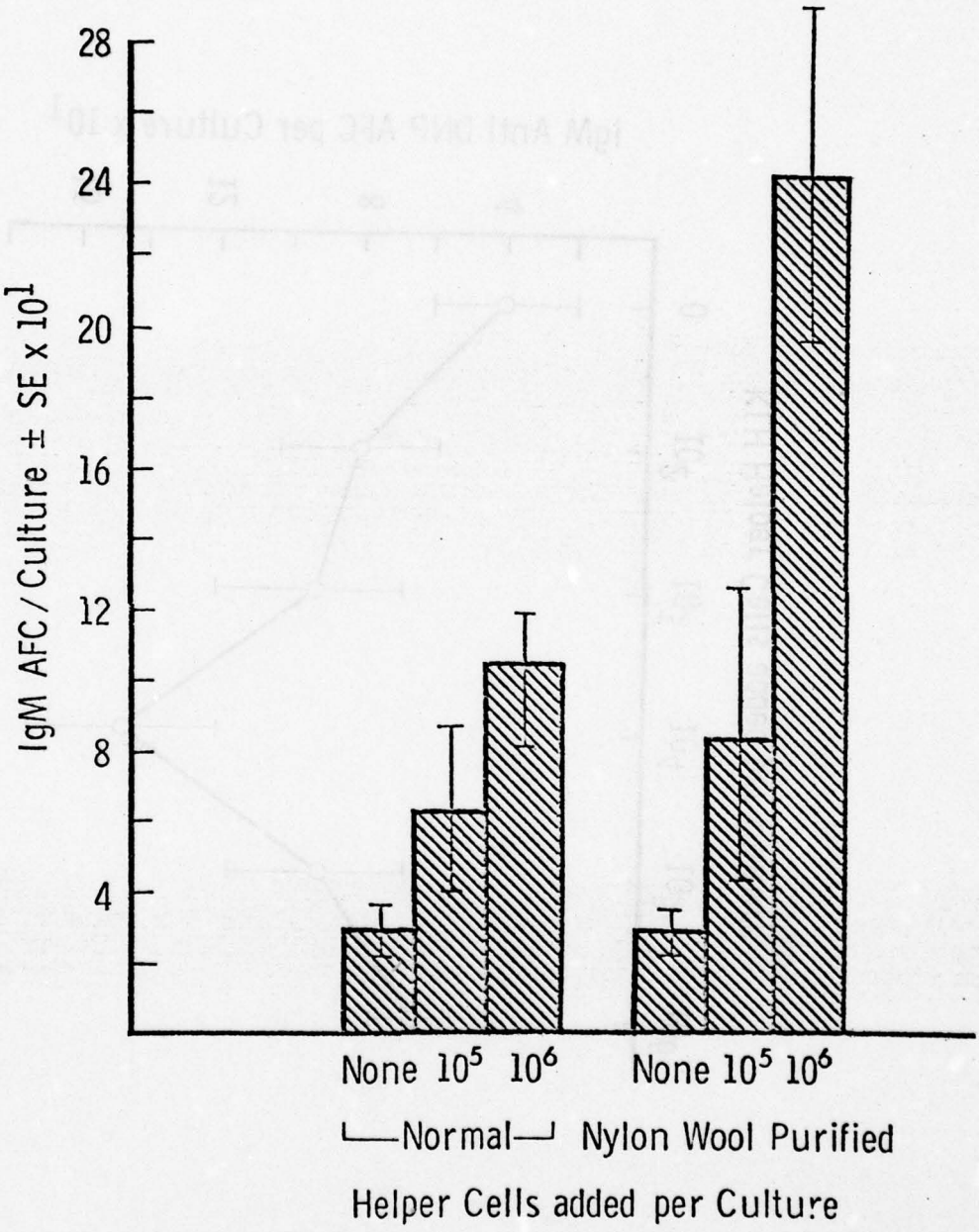

FIGURE 3. EFFECT OF NYLON WOOL PASSAGE ON HELPER CELL ACTIVITY

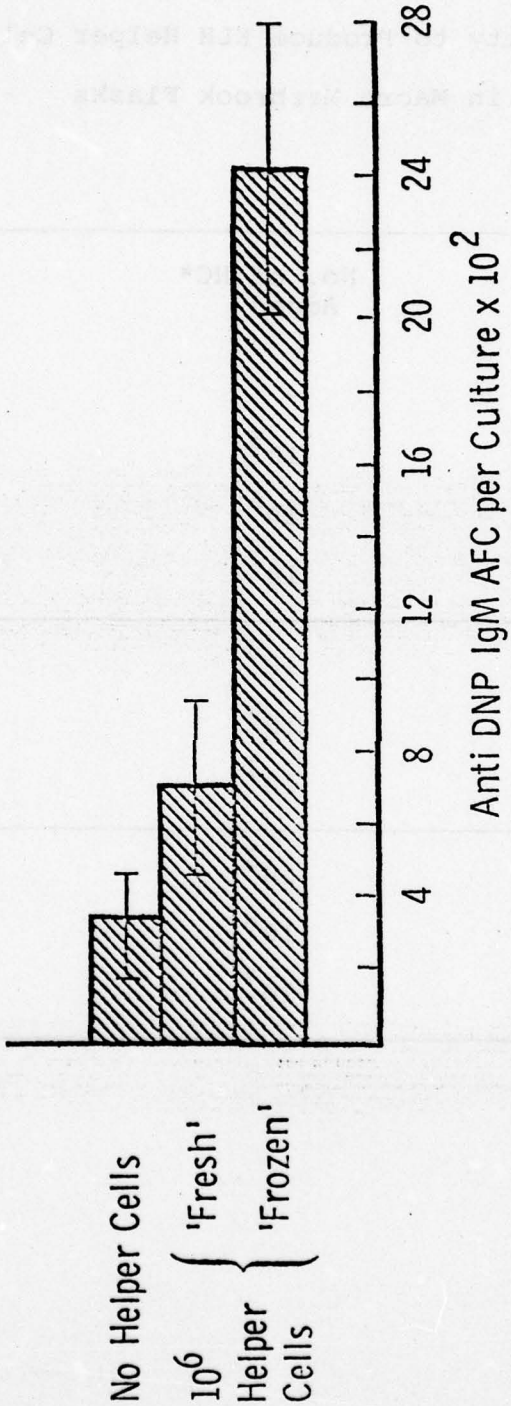

FIGURE 4. ABILITY OF FROZEN HELPER CELLS TO AUGMENT THE ANTIBODY RESPONSE

TABLE 1

Ability to Produce KLH Helper Cells

in Macro Marbrook Flasks

No. of Cells in Flask	No. of HC* Added	IgM AFC** Per Culture ± SE
-	-	63 ± 7
300×10^6	10^5	3 ± 12
	10^6	50 ± 35
	10^7	160 ± 60
600×10^6	10^5	63 ± 18
	10^6	180 ± 32
	10^7	160 ± 42
900×10^6	10^5	70 ± 21
	10^6	77 ± 29
	10^7	153 ± 12

IN "In Vitro Induction and Measurements
of Antibody Synthesis in Man"
Edited by A.S. Fauci and R.E. Ballieux,
Academic Press, 1978 (In Press)

HUMAN ANTIGEN SPECIFIC HELPER T CELL FACTORS IN B CELL RESPONSES

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HUMAN ANTIGEN SPECIFIC HELPER T CELL FACTORS IN B CELL RESPONSES

TABLE OF CONTENTS

I. INTRODUCTION

There are many human diseases with significant derangment of lymphoid function (reviewed by McMichael and McDevitt, 1977). For example some adult acquired hypogammaglobulinemia may be due to suppressor T cells (Waldmann and Broder, 1977). There has thus been an interest in more efficient techniques for measuring human lymphocyte function and many groups of workers have reported attempts to measure human immune responses in vitro (Watanabe et al, 1974; Dosch and Gelfand, 1976, Geha et al, 1977). Recently a number of investigators have reported that human cells can be induced into antibody formation. Thus Fauci and coworkers (1977), have described antibody responses of peripheral blood cells and tonsils to sheep red blood cells while Delfraissy et al. (1977) have described antibody responses to DNP acrylamide beads in an in vitro system.

We have investigated the responses of human T cells chiefly in the generation of antigen specific helper T cells which release antigen specific helper factors (Kantor and Feldmann, 1978; Zvaifler et. al, 1978) and more recently the induction of antigen specific suppressor cells which release specific suppressor factors (unpublished observations).

In this communication we will review our results in the production of specific helper factors (HF) and its relationship to immune response gene function. Since the production of HF is under immune response gene control in the mouse (Munro, et al, 1974; Taussig et al, 1975; Mozes et al, 1975),

assay for HF in humans may permit the definition of a class of human Ir genes, which have been difficult to distinguish by other techniques (Reviewed by VanRood et al, 1977; McMichael and McDevitt, 1977).

II. MATERIALS AND METHODS

A. Animals: C57BL/ICRF, C57BL/6 or C57BL/10 mice were obtained from the Imperial Cancer Research Fund for studies performed in the U.K., and from Jackson Laboratories for studies performed in the U.S.

B. Human Subjects

Healthy volunteers were utilized as donors of peripheral blood lymphocytes (PBL) for these studies.

C. Antigens

(T,G)-A--L, poly-L-(Tyr-Glu)-poly-DL-Ala-poly--L-Lysine was a gift from Dr. E. Mozes, Weizmann Institute, Rehovot, Israel. GAT^{10}, a random terpolymer of 60% L-glutamic acid, 30% L-alanine and 10% L-tyrosine was generously provided by Dr. Paul Maurer, Jefferson Medical College, Philadelphia, Pennsylvania. Keyhole Limpet Haemocyanin (KLH) was prepared and generously donated by Dr. Marvin Rittenberg, University of Oregon Health Sciences Center, Portland, Oregon. Dinitrophenylated (T,G)-A--L, and Dinitrophenylated GAT^{10} was prepared by the method of Sela and Mozes (1966). Conjugates

had approximately 4 groups of DNP per 150,000 daltons. Trinitrophenylated KLH (TNP/KLH) was prepared by Dr. M. Baltz and contained 8 TNP groups per 100,000 daltons of KLH.

D. Tissue Culture Techniques

The techniques used for the induction of helper factors in mice and the assay of these factors using murine spleen cells is described in detail elsewhere (Howie et al, 1977). The methods were similar using either mouse or human cells. In brief, cells were incubated in modified Marbrook Diener flasks along with antigen to produce the factors. Assays of helper factor activity were done using mouse spleen cells plus antigen and helper factor in Marbrook Diener flasks, incubated in an atmosphere of 90% air, 10% CO_2 at 37°C. Media was either MEM or RPMI-1640, with 100 units penicillin per ml, 100 units streptomycin per ml and 2mM L-glutamine. It also contained 5-10% fetal calf serum and 25 mM HEPES or bicarbonate.

E. Human Helper Factor Production

The protocol for human HF production can be seen in the flowchart depicted in Figure 1. In brief, 5 to 10×10^6 PBLs prepared by the method of Boyum (1968) were incubated with 0.1µg KLH, 1.0µg (T,G)-A--L, or 1.0µg GAT^{10} in 1 ml for four days in the Marbrook flask. The cells were harvested, washed once and recultured at the same concentration of viable cells and antigen. Viability at this stage is usually 90%. The

cultures are incubated for 24 hours and the cells centrifuged and the supernatant harvested. The supernatant contains the carrier specific helper factors. The kinetics of HF production is described in detail elsewhere (Zvaifler et al, 1978).

F. Assay of Human HF

The method of assay is schematically represented in Figure 2. In brief, triplicate cultures are set up, each containing a 1 ml mixture consisting of $10x10^6$ murine spleen cells, the appropriate dilution of helper factor, and antigen (0.1µg for TNP-KLH and 1.0µg for GAT^{10}, (T,G)-A--L, DNP-GAT^{10} or DNP-(T,G)-A--L). Controls include antigen alone, DNP polyacrylamide beads or TNP-LPS as a positive thymus independent stimuli, and an equivalent antigen specific mouse helper factor. The cultures are incubated for four days, harvested and the cells assayed for IgM anti-DNP, anti-(T,G)-A--L or anti GAT^{10} antibody forming cells (AFC) per culture using a modified (Strausbach et. al, 1970) Cunningham slide technique. The data is reported as AFC per culture ± the standard error of the mean.

G. Tissue Typing of Donor Lymphocytes

Lymphocytes from donor individuals were tested for HLA-A B, and C determinants using standard serological techniques (Mittal et al, 1968). HLA-D cell typing was done as described previously (Hartzman et al, 1971; Zvaifler et al, 1978)

utilizing both homozygous typing cells and highly discriminant primed lymphocytes in a PLT assay (Sheehe et al, 1975). Using these techniques all currently accepted HLA-A, B, C, and D specificities were tested for; consisting of 20 HLA-A, 22-B and 5-C types as well as HLA-DW1-6, 107 and 108.

III. EXPERIMENTAL RESULTS

A. Production of Antigen Specific Helper Factor from Human PBL

Antigen specific helper factors have been shown to be one of the important components in the induction of thymus dependent antibody responses in mice both *in vitro* or *in vivo* (reviewed by Feldmann et. al, 1977, 1978). Murine helper factor production has been shown to be under genetic control and the factors contain products of the major histocompatibility complex (reviewed by Munro and Taussig, 1975) as well as immunoglobulin variable region markers (Mozes, 1977, reviewed by Feldmann et. al, 1978).

It was thus of interest to determine whether human T cells cooperate with B cells in the same manner, and thus attempts were made to produce antigen specific helper factors from normal peripheral blood leukocytes (Kantor and Feldmann, 1978; Zvaifler et. al, 1978). These have been successful, and helper factors specific for several protein or synthetic polypeptide antigens have been produced from many normal volunteers over the past two years. The function of these

factors has been assayed utilizing the same mouse spleen cell cultures that are used in our laboratories for assaying mouse helper factor activity. There have been several precedents for such cross species action of mediators including both antigen specific and non-specific helper factors (Luzzati et al, 1976; Taussig and Finch, 1977; Farrar et al, 1977).

In general terms, the responses induced by human helper factor has been of the same order of magnitude as those induced by mouse helper factor. However, the dilutions of human helper factor necessary have always been significantly higher (Zvaifler et. al, 1978). This may be due in part to the crossing of species barriers or perhaps because of a lower efficiency of stimulation of normal human T cells. Without other tests for helper factors such as binding assays (which are under development) it is not possible to discriminate between these possibilities although assay of mouse and human helper factor on human B cells would be revealing.

B. Antigen Specificity of Helper Factors

In view of the reports of both antigen specific (Feldmann and Basten, 1972) and non-specific (Schimpl and Wecker, 1972) murine helper factors, it was of interest to ascertain if human helper cells also released both types of activity. Such has indeed been found to be the case (Kantor and Feldmann, 1978; Zvaifler et al., 1978). Higher concentrations of human helper cell supernatant tested (5 to 20%) revealed a

significant degree of non-specific help. This was assayed in several different systems, by augmentative responses to sheep red blood cells, to thymus independent antigens, and to a lesser extent stimulation of the response of non-crossreactive hapten conjugates (Kantor and Feldmann, 1978). Lower concentrations (1% or less) of supernatants of antigen stimulated lymphocytes did not reveal any non-specific activity, but revealed the specific helper effect. This has been tested in two ways by functional means, showing stimulation only with the appropriate antigen (Kantor et al, 1978; Zvaifler et al, 1978) or by immunoadsorbent analysis using antigen coated sepharose columns as shown in Table 1. Supernatants from cells cultured with (T,G)-A--L were incubated with sepharose beads conjugated with (T,G)-A--L or KLH. These supernatants (unbound material), and eluates, (eluted with acid glycine buffer) were tested for their capacity to induce responses to (T,G)-A--L in vitro. $HF_{(T,G)-A--L}$ bound to (T,G)-A--L but not to KLH and was specifically eluded from the immunoadsorbent column. In other experiments HF_{KLH} and HF_{GAT} has been shown to bind specifically only to the appropriate antigen column.

C. Cellular Requirements for Helper Factor Production

In the murine model (reviewed by Feldmann et al, 1977) production of helper cells is dependent on the interaction of macrophages (Erb and Feldmann, 1975) and T cells. Two T cells

were required, an Ly $1^+2^+3^+$ "amplifier" cells and a Ly 1^+ T helper cell (Feldmann et al, 1977). Evidence from other studies (Howie et al, 1977) indicate that it is the Ly 1^+ helper cell that secretes antigen specific helper factor.

In humans, the lymphoid cell populations are less well defined but we have approached the problem of assertaining the cellular requirements for helper factor production by utilizing cell depletion techniques.

Peripheral blood lymphocytes consists of both T and B cells, and to investigate which of these cells are required for the production of helper factors, PBLs were separated into T enriched and a B-monocyte enriched population, using conventional sheep erythrocyte rosette binding techniques (Woody et al, 1975) and separation on hypaque ficoll gradients. Using this method two fractions were prepared, one with 90-95% SRBC rosette positive T cells ($SRFC^+$) while the other, the B monocyte population, contained less than 5% SRBC rosette positive cells ($SRFC^-$). Untreated cells, as well as the "T" and "B-monocyte" populations were used to induce helper factor to KLH. As can be seen in Figure 3, helper factor was produced in the untreated cells and the $SRFC^+$ T cells, but not by the B-monocyte $SRFC^-$ population. Preliminary studies indicate that PBL's depleted of adherent cells fail to produce helper factor, while addition of irradiated PBL's restore this capacity. Taken together the data would suggest

that helper factor production is dependent on both adherent cell and T cells for its production. The 5% or so non-T contaminate in the T cell fractions contain sufficient adherent cell activity for helper factor production.

D. Genetics of Helper Factor Production

The antigens that we have chosen to use for these studies are of two kinds, some like KLH are strong immunogens in essentially all species tested while others like the synthetic polypeptides (T,G)-A--L or GAT^{10} were chosen because in all species tested so far these responses are under the control of MHC linked immune response genes. These have been used to look for evidence for Ir gene function in man and the results obtained so far suggest the existence of analgous Ir genes in man, as detected in other species.

Table II illustrates some of these studies. PBLs from normal volunteers were tested for their capacity to respond to KLH, (T,G)-A--L, or GAT^{10} in parallel cultures on the same pool of PBL. Since all of the individuals tested so far respond to KLH this is used as an internal control for the variable responses to (T,G)-A--L or GAT^{10} where only about 40 to 50% of individuals have responded. Table II shows the response status of eight individuals who have been fully typed for HLA-A, B, C, and D, antigens, the latter by MLC typing. As expected from the very small sample size there is no correlation of responder status with HLA type. This lack of correlation also extends to

the HLA-D region. Here too, DW4 individuals differ in their responsiveness to (T,G)-A--L and GAT[10]. One of these, YO, was HLA homozygous (HLA-A-2, B12, DW4) suggesting that responsiveness to (T,G)AL is not tightly linked to HLA-DW4, although results from one individual should be interpreted with caution. This table also shows the results obtained with two other HLA homozygous individuals which is part of a study using homozygous typing cells to search for HLA association of high low responder status. Family studies have been initiated to search for HLA associations of responder status. These are still preliminary but to date HLA identical sibling pairs have responded in an identical manner to these two antigens.

IV. DISCUSSION

While it is known that human T cells like mouse T cells may be stimulated to release antigen specific helper factors, the characteristics of this material and its relevance to normal processes of immune induction are not yet clear. By most criteria human helper factors are analogous to the mouse helper factor, it is antigen specific, stimulates B cell function, requires macrophages and T cells for its induction (Figure 3, Kantor, F. and Feldmann, M., 1978; Zvaifler et al, 1978).

The serological analysis of mouse helper factor has been fruitful. The factors have been found to contain determinants controlled by the I region of the MHC (Taussig et al, 1975) as well as immunoglobulin like determinants (Feldmann and Basten,

1972; Howie and Feldmann, 1977) which may be related to immunoglobulin variable regions (idiotype) markers (Mozes, 1978). Such information is not yet established in the humans, but there is preliminary evidence for Ia like determinants (unpublished observation). Because of the difficulty in identifying Ir genes in humans (reviewed in VanRood et al, 1977) attempts were made to use these techniques as an assay for the human Ir genes which may control helper factor production. So far the reproducible identification of low responders to particular antigen has been reminiscent of the animal findings, but the genetic analysis is incomplete. Each batch of factor needs to be repeatedly assayed and a given individual tested several times to ensure firm classification of responder status. In each experiment for preparation of helper factor the response to KLH is used as a control of the adequacy of the test as all individuals so far tested have responded.

Family studies using typed individuals should determine whether the response to (T,G)-A--L and GAT^{10} segregates with the HLA complex. The limited studies so far were compatible with this notion. Use of homozygous typing cells to produce factors may permit a closer identification of the helper factor and its genetic origin.

It may be questioned whether an assay of human helper factor on mouse spleen cells reflects a normal human T cell function. So far we have no evidence which suggests otherwise. Only responder mouse spleens respond to human helper factor

thus indicating that it follows the same pathway of stimulation as mouse helper factor. Responses are of the same magnitude and kinetics and there is no evidence of polyclonal activation at the appropriate dilution of helper factor. Testing of human helper factor in vivo, in irradiated mice also showed similarities to stimulation with mouse helper factor (Zvaifler et. al., 1978). However assay of human helper factors on human cells would be desirable and attempts to do this are in progress.

V. ACKNOWLEDGEMENTS

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TABLE 1

ANTIGEN SPECIFICITY OF HUMAN HELPER FACTOR

FACTOR	IMMUNO-ABSORBENT	ANTIGEN	ANTI DNP IgM AFC PER CULTURE ± SE	
			FILTRATE	ELUATE
-	-	-		50 ± 25
-	-	DNP-PAA		867 ± 171
-	-	DNP-(T,G)-A--L		53 ± 3
Human (T,G)-A--L	-	"		267 ± 39
Human (T,G)-A--L	(T,G)-A--L	"	63 ± 23	237 ± 12
Human (T,G)-A--L	KLH	"	250 ± 52	40 ± 23

Helper Factor Donor NZ; Mice C57BL, Response Day 4, of 3×10^6 cultured cells.

TABLE II

HISTOCOMPATIBILITY TYPE AND RESPONDER STATUS

OF NORMAL HUMAN SUBJECTS

DONOR	RESPONDER STATUS			HISTOCOMPATIBILITY LOCUS			
	KLH	(T,G)-A--L	GAT^{10}	HLA-A	HLA-B	HLA-C	HLA-D
MF	+	+*	0*	3/W26	7/W38	- / -*	- / DW6
NAM	+	0	0	2/W24	5.1/8	- / -	- / DW3
UK	+	0	+	1/11	8/W40	W3	DW3/DW5
JW	+	0	+	2/28	12/W40.2	W5	- / DW4
NZ	+	+	0	29/W33	5.1/14	- / -	- / -
YO	+	+	0	2/2	12/12	- / -	DW4/DW4
LA	+	+	0	2/2	5/5	- / -	DW1/DW1
GR	+	+	0	9/9	12/12	- / -	DW1/DW1

* + = high responders; 0 = low responders; - = not detected.

LEGENDS

Figure 1. Flow chart depicting the method used for production of human antigen specific helper factors.

Figure 2. Flow chart depicting the method used for assaying human helper factor activity.

Figure 3. Ability of untreated PBL, $SRFC^+$ (T cells) and $SRFC^-$ (B cells and monocytes) populations to produce helper factor.

Figure 1

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PREPARATION OF ANTIGEN SPECIFIC
HUMAN HELPER CELL FACTOR

$5\text{-}10 \times 10^6$ NORMAL PBL's per 1 ml CULTURE+
ANTIGEN (KLH, (T,G)-A--L, GAT10)

↓

CULTURE 4 DAYS IN A MODIFIED MARBROOK CHAMBER

↓

HARVEST AND COUNT CELLS

↓

RECULTURE AT $5\text{-}10 \times 10^5$ VIABLE CELLS PER ML
WITH SAME AMOUNT OF ANTIGEN FOR 24 HOURS

↓

HARVEST THE CULTURES AND CENTRIFUGE THE CELLS.
COLLECT SUPERNANT AND FILTER THROUGH 0.22μ MILLIPORE FILTER.
STORE AT $-70^{\circ}C$

Figure 2

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ASSAY OF HUMAN HELPER FACTORS

NORMAL MOUSE SPLEEN CELLS + ANTIGEN + DILUTIONS OF HF

↓

INCUBATE FOR 4 DAYS IN A
MODIFIED MARBROOK CHAMBER

↓

HARVEST CELLS

↓

ASSAY FOR ANTI-DNP, (T,G)-A--L or GAT^{10}
IgM AFC PER CULTURE

Figure 3

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