AD-A050 787 OFFICE OF NAVAL RESEARCH LONDON (ENGLAND) F/G 6/5
ANNUAL CONFERENCE OF THE INTERNATIONAL SOCIETY FOR EXPERIMENTAL--ETC(U)
NOV 77 R I WALKER

UNCLASSIFIED ONRL-C-10-77 NL

| OF |
AD
A050787

END
DATE
FILMED
4-78
DDC

AD A050787

2

ONR LONDON CONFERENCE REPORT

ONRL C-10-77

(12)

OFFICE OF NAVAL RESEARCH

BRANCH OFFICE LONDON ENGLAND

DDC FILE COPY

(6) ~~SIXTH~~ ANNUAL CONFERENCE OF THE INTERNATIONAL SOCIETY FOR EXPERIMENTAL HEMATOLOGY, BASEL, SWITZERLAND, 28-31 AUGUST 1977. (6th),

(9) Conference rept,

(10) R.I. WALKER, LCDR, MSC, USN*

(11) 21 NOV~~EMBER~~ 1977

(12) 9 p.

*Armed Forces Radiobiology Research Institute, Bethesda, MD

(14) ONRL-C-10-77

DDC RECEIVED MAR 7 1978 A

THIS REPORT IS ISSUED FOR INFORMATION PURPOSES ON THE UNDERSTANDING THAT IT IS NOT A PART OF THE SCIENTIFIC LITERATURE AND WILL NOT BE CITED ABSTRACTED OR REPRINTED

UNITED STATES OF AMERICA

THIS DOCUMENT HAS BEEN APPROVED FOR PUBLIC RELEASE AND SALE; ITS DISTRIBUTION IS UNLIMITED.

265 000 JOB

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER ONRL C-10-77	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) SIXTH ANNUAL CONFERENCE OF THE INTERNATIONAL SOCIETY FOR EXPERIMENTAL HEMATOLOGY, BASEL, SWITZERLAND, 28-31 AUGUST 1977		5. TYPE OF REPORT & PERIOD COVERED CONFERENCE 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) R.I. WALKER, LCDR, MSC, USN, Armed Forces Radiobiology Research Institute, Bethesda, MD		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Office of Naval Research, Branch Office, London Box 39 FPO New York, NY 09510		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE 21 November 1977 13. NUMBER OF PAGES 8
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

ACCESSION for
NTIS White Section
DDC Buff Section
UNANNOUNCED
JUSTIFICATION
BY
DISTRIBUTION/AVAILABILITY CODES
Dist. AVAIL. and/or SPECIAL

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

MACROPHAGE
GRANULOCYTE
MYELOPOIESIS
HLA-ANTIGENS
PLATELET
REGULATION

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report summarizes a meeting held in Basel, Switzerland (August 1977) in which over 300 scientists from Europe, United States, and several other countries interchanged current information regarding clinical and laboratory experience in hematology. The presentations concerned regulatory mechanisms behind cell proliferation and functional aspects of mature cells. Applications of these basic science principles to treatment of hematologic disorders in human and animal models were described.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-014-6601 |

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

SIXTH ANNUAL CONFERENCE OF THE INTERNATIONAL SOCIETY FOR EXPERIMENTAL HEMATOLOGY, BASEL, SWITZERLAND, 28-31 AUGUST 1977

The Sixth Annual Conference of the International Society for Experimental Hematology was held in the convention center of Sandoz, Ltd., in Basel, Switzerland. As in previous years, the meeting ran from Sunday through Wednesday (28-31 August 1977), but the composition of the presentations was somewhat different. The Conference chairman (Dr. Bruno Speck, Kantonnspital, Basel) told me that he was trying to achieve a meeting of broader interest than had been presented in previous years. He was largely successful.

Over 300 scientists, principally from the US and Europe, participated. The 260 presentations consisted of plenary lectures, symposia, and workshops. Selected plenary lectures and symposia will probably be included in a hard-backed book to be published in about a year. My only criticism of this meeting is in relation to the workshops. In order to accommodate as many participants as possible, the workshops consisted of three simultaneous sessions of talks averaging about five minutes each. This made it difficult to schedule attendance at different workshops and limited the amount of information that could be presented. Poster sessions could probably replace these short oral presentations, thereby permitting a more effective dissemination of information.

The Conference began with three plenary lectures. Recent developments in histocompatibility matching for bone-marrow transplantation were discussed by J.J. van Rood (University Hospital, Leiden, the Netherlands). Graft rejection and graft-versus-host (GVH) disease are major stumbling blocks for a successful outcome in bone-marrow transplantation for aplastic anemia. This problem is thought to be due to incompatibility of donor and recipient for determinants coded for by genes outside the HLA supergene. The author suggested several approaches to solve the problem of graft rejection and GVH disease: identification and matching of non-HLA determinants and nonspecific or specific immunosuppression of donor and/or host.

Clinical experience in treatment of aplastic anemia by bone-marrow transplantation was next discussed by R. Storb (Fred Hutchinson Cancer Research Center, Seattle, WA). Seventy-three patients, 2-67 years of age, with advanced aplastic anemia were given marrow transplants from HLA-identical siblings following 300 mg of cyclophosphamide/kg or 1000-rads total body irradiation. Although mortality was high (approximately half), bone-marrow grafting was found to be an effective treatment of severe aplastic anemia. Marrow-graft rejection was a major problem and was correlated with two factors: (1) detection of recipient sensitization against donor cells, and (2) a low marrow-cell dose.

The immune pathogenesis of aplastic anemia was described by B. Speck (Kantonsspital, Basel). Marrow grafts were studied as a means of correcting the defect at the level of the pluripotent stem cell which is thought to cause aplastic anemia. Speck's group tried to produce split hemopoietic chimerism without GVH by grafting one HLA haplotype mismatched, MLC positive marrow after ALG conditioning in patients without matched donors. The authors conclude that allogeneic marrow transfusion is needed for the autochthonous marrow reconstitution, but in clinical aplastic anemia they did not know if vigorous immunosuppression alone could give similar results.

A symposium concerning hemopoiesis followed the plenary lectures. T.M. Dexter (Christie Hospital, Manchester, England) reported on stem-cell kinetics in long-term bone-marrow cultures. Their group developed a culture system where pluripotential hemopoietic stem cells (CFU-s) and granulocyte precursor cells (CFU-c) can be maintained for several months *in vitro*. Cycling and self-renewal characteristics of CFU-s and CFU-c in bone-marrow cultures were investigated by use of different feeding regimens (depopulation of half the growth medium). After each feeding there was an initial burst of proliferative activity of CFU-s followed by a low cycling stage.

The occurrence of the H-2 antigens on the hemopoietic stem cell was investigated by G.V. den Engh (TNO, Rijswijk, the Netherlands). The H-2 complex codes for three groups of membrane components that are expressed independently and can be detected serologically. The H-2K and H-2D antigens are found on the surfaces of most cells, but the third region, H-2la, seems to be typical for B-cells. The presence of H-2 antigens was used as a tool to study pathways of differentiation from stem cells to lymphocytes.

Fifteen workshops consisting of short presentations were also included in the meeting. Because of the difficulties of attendance presented by three simultaneous sessions, I will summarize only those presentations I was able to attend.

Two papers were presented by D.H. Pluznik's group (Bar-Ilan Univ., Ramat-Gan, Israel) concerning lipopolysaccharide (endotoxin) effects on cell proliferation. Bacterial lipopolysaccharides (LPS) are B-cell mitogens which induce release of colony-stimulating factor (CSF) from lymphocytes. CSF is a humoral factor necessary for the development of macrophage/granulocyte colonies. The cellular requirements for generation of CSF were studied. LPS-stimulated peritoneal macrophages produce large amounts of lymphocyte-activating factor(s) (LAF), while unstimulated macrophages produce insignificant amounts of the factor(s). Large amounts of CSF are generated by nonadherent spleen cells when LAF is added to the culture. Thus generation of CSF by lymphocytes is mediated via a soluble factor transferred from macrophages to lymphocytes. Further studies by the same group concerned the fact that LPS also causes an elevation of serum

interferon (IF). Two inbred mouse strains differing in their sensitivity to LPS were used to study the genetic control of generation of CSF and IF. It was found that the ability to generate serum CSF and IF is controlled by single autosomal-dominant genes.

LPS is known to modulate myelopoiesis. F.G. Staber *et al* (CIBA-GEIGY, Ltd., Basel, Switzerland) compared two other bacterial products expected to be found in the intestine (*E. coli* outer-membrane lipoprotein and *E. coli* murine) to endotoxin with respect to their effects on myelopoiesis. They found that the three different cell-wall components are potent inducers of colony-stimulating activity (CSA). In tissues from LPS-resistant C3H/HeJ mice, lipoprotein and murine, but not lipid A (LPS component), elicited proliferation of B lymphocytes and production of CSA. The authors concluded that bacterial cell-wall components of diverse chemical structure exert profound influences on different stages of myelopoiesis.

Another bacterial agent, *Corynebacterium parvum*, was tested for its ability to enhance hematopoietic activity and survival following whole-body irradiation (I. Basic, Univ. of Zagreb, Yugoslavia). *C. parvum* (CP) treatments increased hematopoietic colony-forming activity in mouse spleens and blood, but not in bone marrow, as determined by exogenous spleen-colony assay. Increased hematopoietic activity caused by CP treatment did not protect mice from the consequences of 650-950 rads irradiation. In fact, more mice died in the CP groups. This increased sensitivity to irradiation may correlate with a decrease in numbers of erythrocytes.

The study of stimulators and inhibitors of hematopoietic activity is complicated by the lack of definition of specific factors involved. Biochemically identified and pure preparations are generally not available. Isolation of colony-stimulating factors from human leukocyte-conditioned medium was described by G. Wagemaker (TNO, Rijswijk, the Netherlands). Two factors with molecular weights of 17,000 and 45,000 daltons were found which stimulate the formation of granulocytic colonies in methylcellulose cultures of human, monkey, and rat cells, but not of mouse bone-marrow cells. A colony-stimulating activity has also been isolated from human lung tissue (W. Hinterberger, Univ. of Vienna, Austria) which may contribute to humoral regulation of myelopoiesis in man. Acid mucopolysaccharides may also play an essential role in developmental processes and regulation of cellular proliferation (R.E. Ploemacher, Erasmus Univ., Rotterdam, the Netherlands). These compounds may inhibit the erythroid but (in the same concentrations) not the granuloid differentiation of bone-marrow-derived hemopoietic cells in an *in vitro* culture system. Mature granulocytes and prostaglandin E_1 may also be inhibitors of granulopoiesis *in vivo* (J.M. Goldman, Hammersmith Hospital, London, England). A crude leukocyte extract was also described which had an inhibitory effect on granulocytic cells of bone marrow (N. Stojanovic, Institute for Medical Research, Belgrade, Yugoslavia).

Canine cyclic hematopoiesis has been a useful model for studying regulation. The proliferation capacity of marrow of dogs with cyclic hematopoiesis (CH) equals or exceeds that of normal dog marrow cultured concomitantly (J.B. Jones, Univ. of Tennessee, Knoxville). There is little chance, based on Jones' work, that competition for a limited number of stem cells is the underlying defect in canine CH. The nature of the defect has not been elucidated.

Functional aspects of mature leukocytes were also covered in this meeting. H.U. Keller (Univ. of Bern, Switzerland) investigated whether locomotion and chemotaxis are controlled by distinct mechanisms or whether chemotaxis is just some form of biased locomotion. These functions were found to be regulated independently. Chemokinetic factors such as human serum albumin or fibrinogen have no chemotactic activity, while chemotactic factors such as preparations of serum peptides containing classical anaphylotoxin exhibit no significant chemokinetic activity.

Considerable evidence is accumulating that granulocytes play important regulatory roles during inflammation. In my report to this Society, I described the phenomenon that i.p. $ZnCl_2$ treatment promotes trapping of i.p. endotoxin within the peritoneal cavity. This trapping kept lethal amounts of endotoxin from entering the bloodstream. However, survival was not enhanced in irradiated mice although peritoneal trapping was similar to that seen in unirradiated animals. Survival in unirradiated animals may be due to the zinc-induced influx of granulocytes into the peritoneal cavity. One can speculate that the increased efficacy of antibiotic treatment of sepsis when granulocyte transfusions are also used may be due in part to the regulatory function of these cells.

Several papers in the same workshop dealt with granulocyte- and platelet-replacement therapy. S. Bhaduri (Univ. of Ulm, Germany) treated 22 patients of acute leukemia with granulocytopenia and severe infection with granulocyte transfusions in addition to bactericidal antibiotics. Cells were collected by continuous-flow centrifugation as well as filtration leukapheresis. Patients were given an average daily dose of 3×10^{10} granulocytes, and over half responded favorably. Reduction of infection with prophylactic-granulocyte transfusions following bone-marrow transplantation was also reported (R.A. Clift, Univ. of Washington, Seattle).

The problem of HLA-antibody production in response to platelet transfusions was studied (J.G. Eernisse, University Hospital, Leiden, the Netherlands). This response became a problem after transfusion of about 20 units of platelets. These preparations are usually contaminated with white cells, and this contamination may be responsible for HLA-antibody formation.

The increased susceptibility to infection in aplastic anemia may be caused not only by the paucity of phagocytic cells, but also by a functional

defect of leukotaxis (S.G. Pahwa, Sloan-Kettering Institute, New York). A return to normal of polymorphonuclear leukocyte and monocyte chemotaxis following bone-marrow transplantation may represent a good prognostic sign.

For granulocyte-transfusion therapy to achieve its clinical potential, suitable means for preservation and long-term storage of these cells must be found. J.E. French (AFRRI, Bethesda, MD) studied liquid and freeze-preserved dog polymorphonuclear granulocytes (PMNG) by ultrastructural analysis. The AFRRI group found that dog PMNG differ in morphology according to the method of isolation and storage medium but do survive to a remarkable degree. Human PMNG are not so resistant to these manipulations, and the author suggested that study of human and dog PMNG differences could provide insight into developing successful preservation methods.

During the immunosuppressed states induced by immunologic manipulations such as bone-marrow transplantations, individuals are extremely sensitive to infection. There is considerable interest in the use of gnotobiotic techniques to shield these individuals from infection. Mice with spontaneous T-cell leukemia were given oral antibiotics to eliminate endogenous infection and maintained in horizontal laminar air-flow (LAF) units to prevent exposure to exogenous sources of infection (R.L. Truitt, Mount Sinai Medical Center, Milwaukee, WI). Leukemic mice had higher survival rates following adoptive immunotherapy with H-2 incompatible donor cells when treated with antibiotics and kept in germ-free environments. There is evidence that human patients with aplastic anemia transplanted in LAF units have a delayed onset of GVH disease and a better survival (C.D. Buckner, Univ. of Washington, Seattle).

The mononuclear phagocytic system was the subject of several reports. Malignant diseases and immunostimulatory manipulations for treatment of malignoma may evoke an accumulation of macrophages in the tissue, the majority of them being recruited from blood monocytes (G. Meuret, Dept. of Internal Medicine, St. Gallen, Switzerland). Immunostimulation by BCG effects a transitory increase in monocyte production.

Macrophage cytotoxicity for tumor cells may require close contact between the attacker macrophage and the target tumor. W.F. Piessens (Harvard Medical School, Boston, MA) determined the ability of activated (with macrophage-activating factor or endotoxin) macrophages to bind tumor cells as compared to unactivated macrophages. Activated phagocytes did bind more tumor cells than normal. These results support the hypothesis that tumor cell-killing by activated macrophages involves cell-to-cell contact.

The alveolar macrophage is believed to have most of the properties of other tissue macrophages, but because of its unique environment, it has developed certain morphological and functional peculiarities. M.C. Territo (Wadsworth V.A. Hospital, Los Angeles, CA) reported on studies

of chemotactic activity of these cells. Human alveolar macrophages had a low level of chemotactic activity. This could be due to the fact that the cells have already arrived at their final resting place, or it could indicate that chemotactic receptors present on the human monocyte have been used up or blocked as the cell localizes in the lung and matures into the alveolar macrophage.

Production of a new biochemical by macrophages was described by M. Rachmilewitz (The Hebrew University-Hadassah Medical School, Jerusalem, Israel). Stimulation of the reticuloendothelial system (RES) of mice was found to cause increased levels of transcobalamin II (TCII). This substance was found to be produced and released only by adherent cells (macrophages). Increased TCII levels have been associated with clinical conditions characterized by proliferation of the RES, such as Gaucher disease and in the active stage of collagen diseases.

Additional symposia were held later in the meeting. J. Kurland (Sloan-Kettering Institute, New York) discussed the capacity of the mononuclear phagocyte (MO) to elaborate opposing biological regulators of *in vitro* granulopoiesis. Accumulation of MO-derived CSF in its own local milieu induces the elaboration of prostaglandin E which directly opposes the stimulatory action of CSF. This central surveillance and effector role of the MO thereby represents a self-regulating unit which functions to prevent excessive myelopoiesis associated with elevation of CSF.

M. Jeannet (Cantonal Hospital, Geneva, Switzerland) reviewed the subject of histocompatibility testing for bone-marrow grafting. Bone-marrow transplantation from an HLA-identical sibling is an effective form of therapy for patients with severe aplastic anemia and immunodeficiency. However, in spite of HLA compatibility with the donor, approximately one-third of the patients with aplastic anemia reject their grafts, and graft-versus-host disease occurs frequently. Histocompatibility antigens outside the HLA complex may therefore be important in bone-marrow grafting. Among the tests commonly used for the selection of a compatible donor, HLA typing and the mixed lymphocyte-culture test appear to be the most important at the present time. *In vitro* tests of humoral and cellular immunity, such as the LDA and CML assays, may also yield valuable information on the state of sensitization of the recipient to the donor non-HLA antigens. In the future it may become necessary also to match the donor and recipient for non-HLA antigens present on granulocytes, lymphocytes or platelets, in order to avoid marrow rejection or severe graft-versus-host reactions.

A methodological report concerning the problem of testing for the non-HLA antigens mentioned above was presented later (C.P. Engelfriet, Central Laboratory of the Netherlands Red Cross Transfusion Service, Amsterdam). An immunofluorescent technique applicable to granulocytes was described, and the sensitivity of this technique in comparison with the leukoagglutination test (LAT) and the granulocyte-cytotoxicity technique (GCT) was

discussed. Allo- and autoantibodies against granulocyte-specific antigens that do not react in either the LAT or GCT can be detected with this method.

The role of macrophages in the defense mechanisms against infection and malignancy was discussed by A. Cruchaud (Cantonal Hospital, Geneva, Switzerland). Macrophages occupy two key positions in a chain of events that constitute the defense of organisms against microorganisms and tumor cells. First they take up antigens coated with antibody and complement thanks to the presence, on their plasma membranes, of receptors for Fc, C3b, and C3d. Immunogenic moieties subsequently located on the macrophage plasma membrane can trigger competent lymphocytes to develop an immune response. On the one hand, B lymphocytes transform into antibody-synthesizing plasma cells. Antibodies combine with antigens to form immune complexes which fix complement. The activation of the complement sequence produces by-products that are chemotactic for phagocytic cells. On the other hand, T lymphocytes secrete a number of agents called lymphokines that attract, immobilize, and activate macrophages.

Second, activation of macrophages and opsonization of microorganisms by antibodies result in more effective phagocytosis and enhanced bactericidal activity. Also, macrophages coated with specific arming factor will kill microorganisms and tumor cells by contact. These events are accompanied by the release of lysosomal enzymes which are mostly acid hydrolases. These participate in tissue degradation in the environment of macrophages and in inflammatory processes. The initial phagocytic process thus generates the collaboration of other cells, mostly lymphocytes, which secrete factors that in turn amplify the phagocytic, bactericidal, and cytotoxic capacity of macrophages.

It is this author's opinion that the International Society for Experimental Hematology will continue to grow and broaden its appeal. Understanding the interplay between peripheral components of the immunologic-inflammatory systems and their source in the marrow will provide a key to treatment of diseases known to be hematologic in nature as well as many that presently are not. It is hoped that the summary of the reports presented above will give some idea of the scope of the subject of experimental hematology.