1981

Characterization Of Human Suppressor Cells.

Arabella Bukacek Tilden
University of Alabama at Birmingham

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CHARACTERIZATION OF HUMAN SUPPRESSOR CELLS

The University of Alabama in Birmingham PH.D. 1981

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CHARACTERIZATION OF HUMAN SUPPRESSOR CELLS

by

ARABELLA BUKACEK TILDEN

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Microbiology in The Graduate School of The University of Alabama in Birmingham

BIRMINGHAM, ALABAMA

1981

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Charles Balch who provided encouragement and guidance throughout these studies. I would also like to thank Judy Williams, Beth Kennedy, and Linda Hudson for their ready assistance on innumerable occassions. A special thanks goes to Patsy Dougherty for both her "helping hand" and "shoulder to cry on".

ABSTRACT OF DISSERTATION

GRADUATE SCHOOL, UNIVERSITY OF ALABAMA IN BIRMINGHAM

Degree Doctor of Philosophy Major Subject Microbiology

Name of Candidate Arabella Bukacek Tilden

Title Characterization Of Human Suppressor Cells

In the first part of this study the induction and cell surface phenotype of human suppressor T lymphocytes were examined. Human T cells and T cell subsets were separated on the basis of cell surface phenotype and tested for Concanavalin A (Con A) induced suppression of mixed lymphocyte reactions (MLR). Early experiments indicated that adherent cells were required for the induction of suppressor T cell activity. Several markers expressed on T cell subsets were studied in order to determine whether these were markers specific for suppressor T lymphocytes. These markers included the FcIgG receptor, a DR antigen and a 16,000 MW antigen. These markers were found not to be unique markers of suppressor T cells.

In the second part of this study a group of patients (melanoma), previously shown to exhibit decreased immunocompetence, were examined for abnormalitites in suppressor cell activity. A depressed immune response in melanoma patients was confirmed by the

finding that peripheral blood mononuclear cells (PBMC) from 32 out of 33 of these patients displayed abnormally low mitogen responses. These responses were significantly enhanced when indomethacin, a prostaglandin synthetase inhibitor, was added to PBMC cultures from melanoma patients. The mitogen response of T cells from melanoma patients was equivalent to that of normal controls, and there was no enhancement of this response in the presence of indomethacin. These results suggested that prostaglandin mediated suppression by monocytes was responsible for abnormal mitogen responses in melanoma patients. However, three sets of experiments excluded this possibility: (1) Prostaglandin production from PBMC was the same in melanoma patients and normal controls, (2) Lymphocyte sensitivity to prostaglandin for melanoma patients was essentially the same as normal controls, since exogenous doses of prostaglandin inhibited the mitogen responses to the same degree and (3) An experimental drug (RO-205720) that specifically inhibits prostaglandin production did not increase mitogen responses in melanoma patients. Thus it appears that the decreased immunocompetence of melanoma patients is due to a defect in immunoregulation by monocytes and is unrelated to prostaglandin mediated suppression.

Abstract Approved by: Committee Chairman Charles V. Balch M.D.

Program Director Brenda J. Johnson

Date 5/29/81 Dean of Graduate School Kenneth Rogers

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LIST OF ABBREVIATIONS

AET	2-amino-ethylisothiouronium bromide
AMT	Anti-monkey thymocyte serum
AMT/M4	Anti-monkey thymocyte serum adsorbed with Molt-4 cells.
BCG	Bacillus - Calmette Guerin
BSA	Bovine serum albumin
Con A	Concanavalin A
C. parvum	Corynebacterium parvum
CPM	Counts per minute
$Cr\ Cl_3$	Chromium chloride
ER	Erythrocyte rosette
FACS	Fluroescence activated cell sorter
FcIgG	The fragment crystallizable portion of IgG
FcIgM	The fragment crystallizable portion of IgM
FCS	Fetal calf serum
MASH	Multiple automated sample harvester
MLC	Mixed lymphocyte culture
MLR	Mixed lymphocyte culture
MW	Molecular Weight
NK	Natural Killer
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PG	Prostaglandin

LIST OF ABBREVIATIONS (Continued)

PGE_2	Prostaglandin E_2
PHA	Phytohemagglutinin
PWM	Pokeweed mitogen
RBC	Red blood cell
RIA	Radioimmunoassay
SRBC	Sheep red blood cell
T_M	The subpopulation of T cells with receptors for FcIgM
T_G	The subpopulation of T cells with receptors for FcIgG

BACKGROUND

Suppressor Cells

Suppressor lymphocyte activity was first described by Gershon and Kondo (1970, 1971) when these investigators found that specific nonresponsiveness could be adoptively transferred by lymphoid cells from tolerant animals. When T cells were eliminated prior to adoptive transfer, the recipients were no longer rendered non-responsive. Thus these investigators introduced the concept of a suppressor T cell in the development of immunological tolerance. Subsequently, suppressor cells have been implicated in virtually all immunologic regulatory systems (reviewed by Waldmann and Broder, 1977). For example, they have been shown to play a role in the control of contact and delayed type hypersensitivity reactions, and in the limitation of antibody responses.

There appears to be a variety of suppressor cells systems rather than a single suppressor mechanism. T cells are the major cell type with suppressor activity. In recent years it has become evident that monocytes are important in immunodepression by virtue of both active suppression and lack of helper function (Nelson, 1976; Kirchner et al., 1975; Goodwin et al., 1977a). B cells have also been implicated as suppressor cells in certain systems (Waldmann and Broder, 1977; Zembala et al., 1977). Furthermore, collaboration between two or more cell types may be necessary for

development or expression of suppressor cell function in some situations. Some suppressor cell activity is antigen specific whereas others are nonspecific. In addition, in some cases suppresssor cell function is genetically restricted and in other cases no genetic barrier to suppression is seen.

Surface Markers of Lymphocytes

Characterization of suppressor cells is facilitated by their expression of particular cell surface antigens or receptors. Several years ago it was illustrated that mouse suppressor T cells express genetically determined cell surface molecules which distinguish them from other functional subsets of T cells. Thus suppressor and cytolytic T cells, in addition to the theta antigen present on all mouse T cells, express Ly 23 antigens, whereas helper T cells express the Ly 1 antigen (Cantor and Boyse 1975a, 1975b, 1976). Suppressor T cells can be further distinguished from cytotoxic T cells by another cell surface molecule coded for in the I region of the mouse major histocompatibility complex (MHC) (Murphy, 1978).

Before the development of monoclonal antibodies, the distinguishing surface markers of human T cells were less well defined than those in the mouse. The first T cell marker discovered was the receptor (designated ER for erythrocyte receptor) that binds sheep red blood cells (SRBC) (Jondal et al., 1972). Owen and Fanger prepared the first heterologous antiserum which recognized human T cell antigens (1976). Several groups have since prepared heterologous antisera which after selective adsorption react

specifically with human T cells or subpopulations of T cells (Balch et al., 1977; Stevens and Saxon, 1978; Evans et al., 1977). Other known markers on human T cells are receptors for the Fc portion of different classes of immunoglobulin (Moretta et al., 1977; Lum et al., 1979). Recently, monoclonal antibodies have defined markers on human T lymphocytes with a precision approaching that of the mouse Lyt alloantisera. However, at the time these studies were begun, a reproducible and definitive phenotype had not been established for functionally distinct T cell subpopulations.

Suppressor Cells and Human Disease

Disorders of suppressor cell systems have been associated with a number of diseases (Stobo et al., 1976; Moretta et al., 1977; Reinherz et al., 1980). For example, a reduction in suppressor cell function has been implicated in the pathogenesis of autoimmune diseases (Horowitz et al., 1977). Other diseases, such as hypogammaglobulinemia and cancer, may be the result of or potentiated by too much suppression (Waldman et al., 1974; Broder et al., 1978). A relative excess of suppressor cell activity has been demonstrated in patients with head and neck carcinomas (Balch et al., 1981), lung carcinoma (Jerrells et al., 1978; Han and Takita, 1980), osteogenic sarcoma (Yu et al., 1977), bladder cell carcinoma (Bean et al., 1977), Hodgkin's lymphomas (Goodwin et al., 1977b) and multiple myeloma (Broder et al., 1975). Furthermore, there is some indirect evidence that immunotherapy in cancer patients might activate suppressor cell activity especially by monocytes. A

greater knowledge of suppressor cells and mechanisms of suppression would lead to better therapeutic strategies in the treatment of immune suppression and autoimmunity.

Objectives of this Study

The ability to identify suppressor cells by surface phenotype would greatly facilitate the modulation of these cells in the treatment of immune disorders. In this study the author examined the phenotype of human suppressor T lymphocytes. In addition, the immunodepression of a group of cancer patients (melanoma) was examined in the context of increased suppressor cell activity.

MATERIALS AND METHODS

Subjects

Normal Donors

Healthy adult volunteers donated from 10 to 250 ml of venous blood for use in these studies.

Melanoma Patients

A total of 55 melanoma patients ranging in age from 24 to 76 were studied. Twenty-eight of the patients had localized disease (Stage I), 17 had regional node metastases (Stage II) while 10 had distant metastases (Stage III). Fifteen patients were receiving non-specific immunotherapy, Bacillus Calmette Guerin (BCG) or Corynebacterium parvum (C. parvum), at the time of study. None were receiving chemotherapy.

Cells

Total mononuclear cells were separated from heparinized blood by Ficoll-Hypaque density centrifugation as described by Balch et al. (1977). The peripheral blood mononuclear cells (PBMC) were washed and resuspended in RPMI 1640 with 20% serum and gentamicin. PBMC were then adjusted to appropriate concentrations and used in

the various assays or further separated into subpopulations before testing.

Antibodies

AMT is a rabbit anti-monkey thymocyte serum prepared in the laboratory of Dr. Charles Balch. AMT is adsorbed to human T cell specificity and fluoresceinated for use in direct immunofluoresceinated assays (Balch et al., 1977). This antiserum has been shown to react with two T cell differentiation antigens having molecular weights of 16,000 and 25,000 daltons by immunoprecipitation techniques (Ades et al., 1978). Reactivity of one antibody type in AMT can be removed by adsorbing the serum with Molt-4 cells, a cultured human T cell line, which express a preponderance of the 25,000 molecular weight (MW) antigen. The resulting antiserum, designated AMT/M4, reacts with only the 16,000 MW antigen and stains 30-40% of PBMC compared to 70-80% recognition by AMT.

A mouse monoclonal anti-human DR reagent was a gift of Dr. Saldono Ferrone. This antibody reacts with framework determinants on all known human DR alloantigens (Indiveri et al., 1980).

OK-T3 is a human T cell specific reagent purchased from Ortho Laboratories. The preparation and specificity of this monoclonal antibody were described by Reinherz and coworkers (1979a).

Lymphocyte Separation Techniques

Isolation of Adherent Cells

5 ml aliquots of Ficoll-Hypaque purified PBMC (at 5 x 10^6 in RPMI 1640 with 10% FCS) were added to 20 x 100mm plastic tissue plates (Corning). The plates were incubated at 37°C for 45 minutes. The nonadherent cells were removed by three washes with warmed PBS. A 0.5% solution of Lidocaine (Invenex) in calcium and magnesium free PBS was added to the plates. The plates were incubated at 4°C for 15-20 minutes. The adherent cells were removed by vigorous pipetting.

Isolation of ER^+ T Cells

One ml aliquots of Ficoll-Hypaque purified PBMC (at 1 x 10^7 cells/ ml in RPMI 1640 with 10% FCS) were added to tubes containing equal volumes of 5% SRBC treated with either neuraminidase or 2-amino-ethylisothiouronium bromide (AET) (Pellegrino et al., 1975). The mixtures were centrifuged at 1,000 rpm at 4°C for 10 minutes, and then incubated on ice for an additional 10 minutes. The pellet was gently resuspended in 5 ml, layered over 2 ml of Ficoll-Hypaque, and centrifuged at 2,000 rpm, at 4°C for 20 minutes. The pellet was again gently resuspended and subjected to another Ficoll-Hypaque purification. The second pellet was resuspended and the SRBC were removed by treatment with ammonium chloride lysing buffer. More than 98% of the pelleted cells were ER^+ cells.

Isolation of $FcIgM^+$ and $FcIgG^+$ T Cells

Ox erythrocytes sensitized with non-agglutinating doses of rabbit IgM or IgG anti-ox erythrocyte were used to rosette ER^+ T cells in order to separate them into $FcIgM^+$ (T_M) and $FcIgG^+$ (T_G) populations. The method employed was that of Moretta et al. (1977). Briefly, isolated T cells were mixed with 2% solutions of sensitized ox RBC. After the appropriate incubations, rosetting and non-rosetting cells were separated by passage over Ficoll-Hypaque. Rosetting cells were always subjected to a second passage over Ficoll-Hypaque to achieve greater purity. Erythrocytes were removed by lysis with amonium chloride lysing buffer.

Chromium Chloride Treatment of SRBC

SRBC were coated with rabbit anti-mouse Ig by treatment with chromium chloride ($CrCl_3$) as described originally by Gold and Fudenberg (1967) and modified by Indiveri et al. (1979). Briefly, one ml of SRBC, washed 3x with saline and resuspended at 10% in saline, were centrifuged and the pellet mixed with 100µl of rabbit antimouse Ig and 100µl of $CrCl_3$ (1 mg/ml in saline) for five minutes at room temperature. The SRBC were then washed 3x times with saline, resuspended at 1% and stored in the refrigerator up to one week. ER^+ T cells, stained for 30 minutes (4°C) with mouse anti-human DR antibody (Indiveri et al., 1980) were mixed with anti-mouse Ig coated SRBC. Rosetting and non-rosetting cells were separated according to the procedure for isolating ER^+ T cells described above.

Separation of Cells with the FACS

Lymphocyte populations were separated in a fluorescence activated cell sorter (FACS) (Becton Dickinson Co., Mountain View, California) as originally described by Herzenberg et al. (1976). The populations were sorted based upon relative fluorescence intensity when stained with fluorescein conjugated antibodies. In some cases lymphoid cells were sorted based on light scattering (size).

Activation of Suppressor Cells

Cell populations to be tested for suppressor activity were adjusted to 2×10^6 cells/ml in RPMI 1640 supplemented with 20% human serum (either autologous or pooled AB sera) and gentamicin. Cell cultures were incubated for 40-48 hours at 37°C in a 5% CO_2 humidified atmosphere both with (test) and without (control) 40 µg/ml Con A. At the end of the incubation period, both control and Con A activated cells were washed with PBS containing 5% FCS, treated with mitomycin-C, and washed three additional times. The cells were counted, resuspended in culture medium, and tested for suppressor activity in mixed lymphocyte cultures.

Mixed Lymphocyte Culture

Standard one-way mixed lymphocyte cultures (MLC) were performed by incubating 2×10^5 freshly isolated responder

lymphocytes with 2 x 10^5 mitomycin-C treated allogeneic lymphocytes for 6 days in RPMI 1640 supplemented with gentamicin and 20% responder serum (or pooled human AB sera) in microtiter plate wells. Cultures were pulsed on day 5 with 0.125uCi ^{14}C-thymidine/well and harvested on day 6 with a multiple automated sample harvester (MASH). Stimulation was measured by counts per minute (CPM) of ^{17}C-thymidine in triplicate cultures.

Suppressor cell activity was determined by adding mitomycin-C treated control and Con A activated cells to the MLC. The control and Con A activated cells were autologous to the responder cells in the MLC. Suppression was calculated by the formula:

$$\% \text{ SUPPRESSION} = \frac{\text{(CPM of the MLC in the presence of cells cultured with Con A)}}{\text{(CPM of the MLC in the presence of control cultured cells)}} \times 100$$

Mitogen Assays

5 x 10^5 PBMC or subpopulations, suspended in RPMI 1640 with 20% FCS and gentamicin, were added to wells of microtiter plates. Different doses of phytohemagglutinin (PHA) or Con A were added directly to wells to achieve the final concentrations indicated in the results. In some experiments cultures were incubated at 37°C with 5% CO_2 for a total of 96 hours, and pulsed with 0.125 µCi/well of ^{14}C-thymidine 16 hours prior to harvesting with a MASH. In other experiments, cultures were incubated for a total of 72 hours, and pulsed with 0.5 µCi/well of 3H-thymidine 8 hours

prior to harvesting. The net CPM of triplicate cultures was calculated as CPM of cells with mitogen minus CPM of cells without mitogen. To test the affect of drugs, simultaneous mitogen assays were performed in the presence of 1 μg/ml of indomethaciin (Sigma) or 20 μg/ml RO-205720 (Hoffman-La Roche). The percent change in the presence of indomethacin or RO-205720 was calculated by the formula shown below:

$$\% \text{ change} = \frac{(x-y)100}{y}$$

where x was the net CPM with indomethacin or RO-205720 and y was the net CPM without drugs.

Radioimmunoassay (RIA) for PGE_2

Prostaglandin E_2 (PGE_2) levels in PBMC culture supernatants were determined in radioimmunoassays (RIA) according to the procedure of Jaffe et al. (1973) as modified by Taffet and Russell (1981). The concentration of PGE_2 in a sample was determined by the competition of cold PGE_2 and ^3H-PGE_2 (New England Nuclear) for a limited number of binding sites on rabbit anti-PGE_2 (Sigma). For each assay a standard curve was set up with the following concentrations of commercial PGE_2 (Sigma); 10 ng/ml, 5 ng/ml, 1 ng/ml, 0.5 ng/ml, and 0.1 ng/ml. One tenth ml samples of standards and unknowns (culture supernatants) were placed in 12 x 75 mm glass tubes. To these were added 0.5ml of ^3H-PGE_2 (7,000-10,000 cpm/0.05ml) and 0.1ml of rabbit anti-PGE_2 (a quantity that binds

50% of the added counts). Control tubes consisted of the following: (1) label and antibody only, to determine maximum binding; (2) label only, to determine background counts.

The tubes were incubated at 4°C for 2-3 hours with occasional shaking. At the end of the incubation, 0.5ml of dextran activated charcoal was added to bind free antigen. After a 5 minute incubation at 4°C, the tubes were centrifuged at 2,000 rpm, 4°C, for 10 minutes. 0.2ml samples of the supernatants were added to 3ml of Aquasol (New England Nuclear) and counted in a scintillation counter. All controls, standards, and unknowns were tested in duplicate. The means of the control values were used to determine the percent inhibition of the other samples according to the formula as shown:

$$\% \text{ Inhibition} = 100 \left(1 - \frac{(\text{Sample} - \text{background})}{(\text{Maximum binding} - \text{background})} \right)$$

Standard curves were constructed by plotting % inhibition vs. log PGE_2 concentration/ml. A linear regression was performed with a Hewlit Packard calculator. The PGE_2 content of the unknowns was calculated by the formula:

$$PGE_2 \text{ concentration} = 10 \left(\frac{\% \text{ inhibition} - y \text{ intercept}}{\text{slope}} \right)$$

CON A SUPPRESSOR CELLS

Introduction

The induction of human suppressor cells by Con A was first described by Shou and coworkers (1976) and Hubert and coworkers (1976). The Con A suppressor cell in these assays was shown to inhibit T cell proliferative responses to alloantigens, PHA, Con A, PPD, and Candida. Other investigators have shown that Con A activated suppressor cells will inhibit pokeweed mitogen (PWM) induced B cell differentiation (Haynes and Fauci, 1977).

Sakane and Greene (1977) demonstrated that the suppressor cell induced by Con A could be found in T cell populations but not in B cell populations. These investigators fractionated human T cells on discontinuous bovine serum albumin gradients. They found that low density resting T cells, which actively proliferated in response to Con A, did not suppress proliferative responses. Conversely, high density resting T cells demonstrated minimal thymidine incorporation in response to Con A yet consistently developed marked suppressor activity for proliferation of B and T cells. These results indicated that the suppressor cell activity induced by Con A could be localized in a subpopulation of T cells and that suppression may be unrelated to cellular proliferation.

In the mouse system the Con A induced suppressor cell bears the same Ly phenotype as other active suppressor T cells (Tse and Dutton, 1977). Therefore the phenotype of the human Con A suppressor cell might define cell surface markers present on all human suppressor T cells. The investigator proposed to use the Con A suppressor cell assay as a means of testing the hypothesis that the human Con A induced suppressor cell belongs to a phenotypically distinct subset of T cells.

At the time these studies were initiated, the cell surface markers of human T lymphocytes and T lymphocyte subsets had not been conclusively defined. Several laboratories, including ours, had developed xenoantisera which reacted with antigens on human T lymphocytes (Balch et al., 1977; Stevens and Saxon, 1978; Evans and Chess, 1977) some investigators had correlated surface marker expression with specific functional activity (Evans and Chess, 1977; Moretta et al., 1977).

In 1977 the most promising markers on human T lymphocytes were the Fc receptors for IgM and IgG. Moretta and colleagues (1977) had reported that functionally distinct subpopulations of human T lymphocytes could be identified and separated on the basis of Fc receptor expression. These investigators found that T cells with receptors for FcIgG (designated T_G), when activated with immune complexes, suppressed PWM induced B cell differentiation. In contrast, T cells with receptors for IgM (designated T_M) exhibited helper activity in B cell differentiation assays.

These studies were designed to use the T cell markers available in 1978, Fc receptors and xenoantisera, to test the hypothesis that human suppressor T lymphocytes express unique surface markers. The results of this study indicated that the available means of identifying and separating T cells were inadequate to define the Con A suppressor cell. The advent of monoclonal reagents to T cell subsets and the pure populations obtained by FACS separation were needed in order to prove the hypothesis that the Con A suppressor cell is confined to a phenotypically distinct T cell subset. Using both these tools, Reinherz and coworkers (1980) later proved this hypothesis. These investigators prepared a monoclonal antibody (designated OKT5) which reacted with an antigen present on 20% of human blood T cells. Upon FACS separation of T cells into $OKT5^{+}$ and $OKT5^{-}$ fractions, they found that Con A inducible suppressor cells were localized in the $OKT5^{+}$ subset. Furthermore, the OKT5 antibody appears to define a human cytotoxic/suppressor T cell subset comparable to the mouse $Ly23^{+}$ subset.

Proposal

Hypothesis: The human suppressor cell induced by Con A belongs to a distinct T cell subset with a unique surface phenotype.

Objective: To identify the Con A suppressor cell by separating T cells into subsets based on surface markers and testing the isolated subpopulations for Con A suppressor cell activity.

Rationale: Suppressor cells in the mouse have been shown to be a phenotypically distinct subpopulation of T lymphocytes and there is evidence for a similar subset in humans.

Results

T_G Cells

The first T cell marker tested for suppressor cell specificity was the FcIgG receptor. T cells with receptors for FcIgG had been shown to exhibit suppressor cell activity in PWM induced B cell differentiation. In addition, T_G cells had a decreased response to Con A in comparison to total T cell populations. Thus T cells with receptors for FcIgG exhibited two properties which suggested that this marker might delineate the Con A suppressor cell.

In initial experiments, PBMC were separated into ER^+ T cells, T_G cells and T_G depleted populations. These subpopulations were cultured with Con A and then tested for suppressor cell activity in MLCs. Figure 1 outlines the experimental protocol for the Con A suppressor cell assay. Early results indicated that Con A activated T_G cells suppressed a MLC by an average of 40% suppression, whereas Con A activated T_G deprived cells exhibited minimal suppressor cell activity with an average of 8% suppression (Figure 2). Thus the Con A induced suppressor cell appeared to reside in the T_G fraction of T cells (Bukacek et al., 1979). Furthermore, these experiments indicated that T cells and T cell subpopulations exhibited less suppressor cell activity than unseparated PBMC.

Figure 1. Scheme for the assay of Con A induced suppression of MLCs.

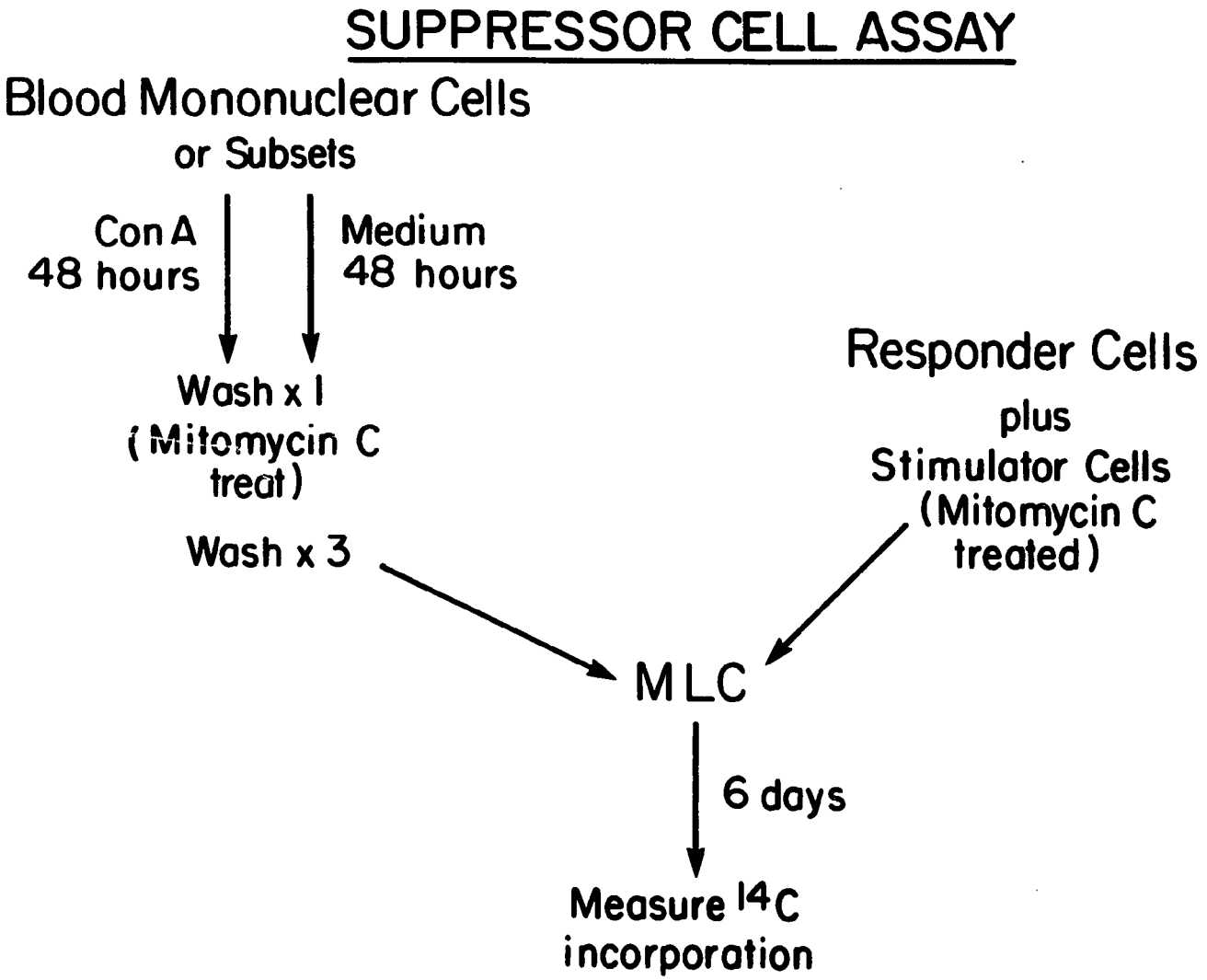
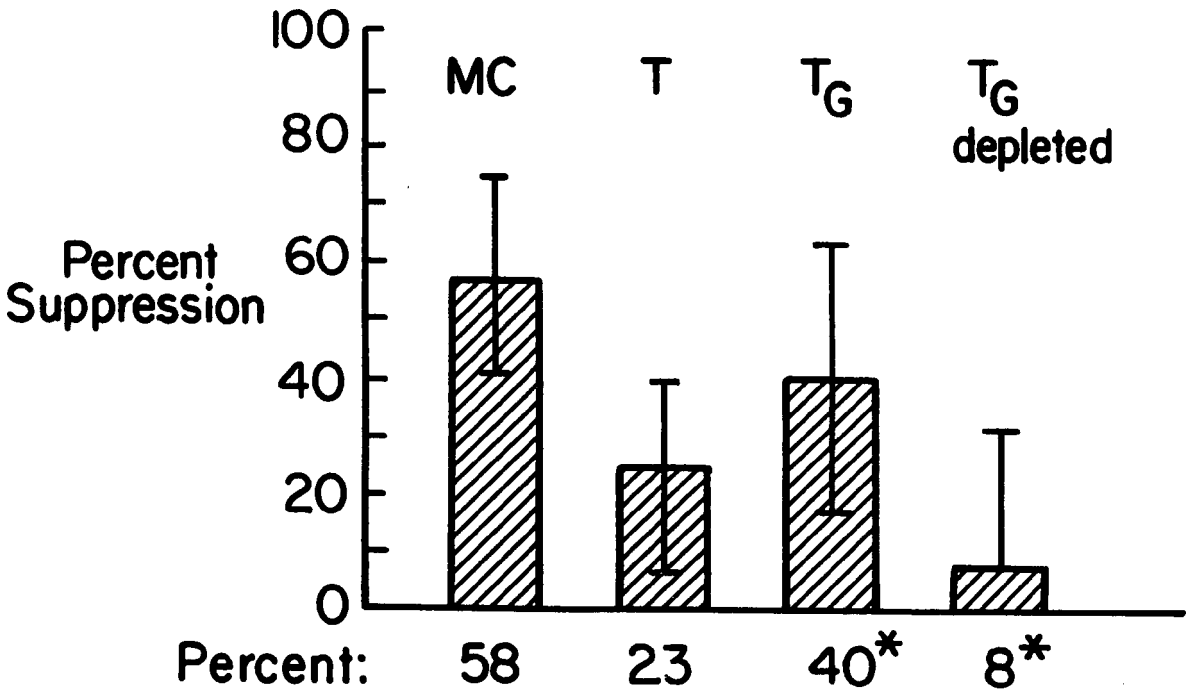

SUPPRESSOR CELL ASSAY
Blood Mononuclear Cells
or Subsets
Con A
48 hours
Medium
48 hours
Wash x 1
(Mitomycin C
treat)
Wash x 3
Responder Cells
plus
Stimulator Cells
(Mitomycin C
treated)
MLC
6 days
Measure 14C
incorporation

Figure 2. Subset analysis of MLC suppression. Comparison of Con A induced suppression of MLCs by total blood mononuclear cells (MC), total T cells, and T_G and T_G deprived T cell subpopulations.

SUBSET ANALYSIS OF MLC SUPPRESSION

(11 experiments)



* $p < 0.01$

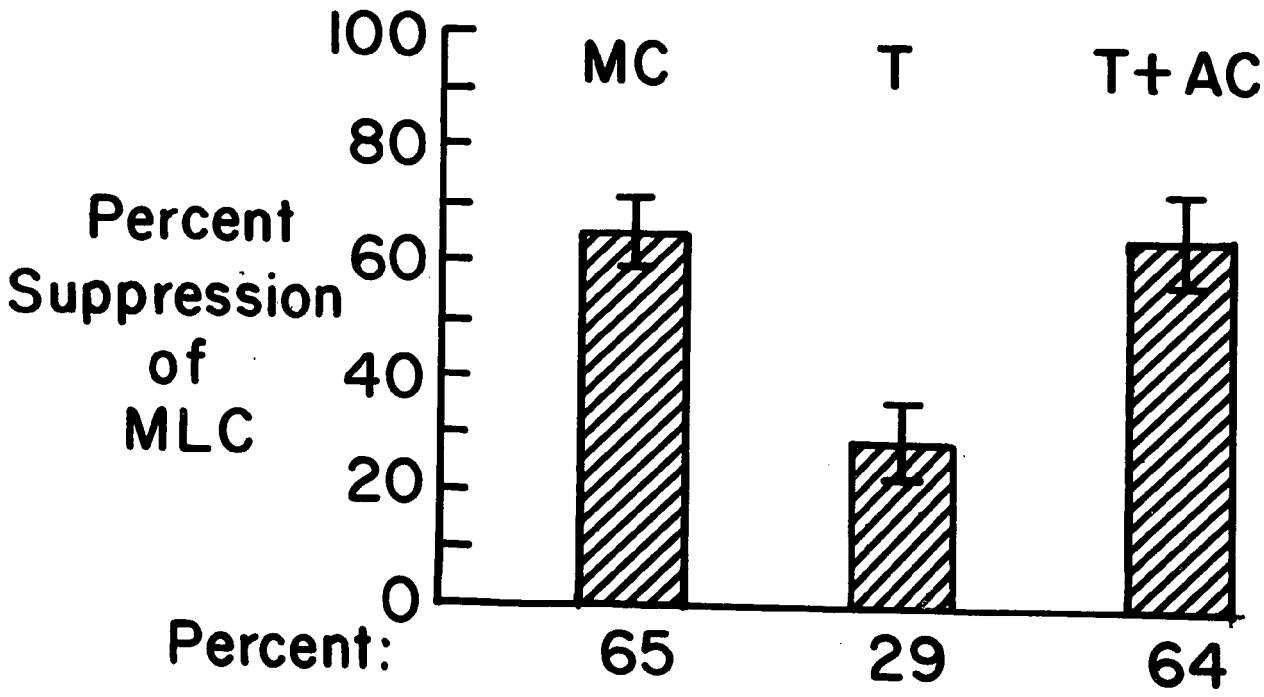
Adherent Cell Requirement

The possibility that adherent cells were required for optimal suppressor cell induction by Con A was examined because it is known that monocytes are required for many T cell functions (Nelson, 1976). To test this possibility adherent cells were isolated and added to ER^+ cells during the Con A culture period. ER^+ T cells cultured with adherent cells exhibited the same amount of suppressor activity as unseparated PBMC whereas ER^+ T cells cultured alone had drastically reduced suppressor cell activity (Figure 3). Different percentages of adherent cells in the T cell cultures were tested to determine the optimal number of adherent cells required for suppressor cell induction. The addition of 5% adherent cells was found to be the most consistent number required for maximum suppressor cell induction but in some experiments the addition of 1% adherent cells was sufficient for induction of maximum activity. Adherent cells alone, when added to MLCs in the concentrations used in suppressor cell induction, did not suppress allogeneic responses. Furthermore, ER^+ T isolated after Con A culture were as efficient as PBMC in suppressing a MLR. Thus the adherent cells themselves did not appear to suppress the allogeneic response but were required for the induction of suppressor activity by T lymphocytes. Additionally, the accessory function of the adherent cells was radioresistant, since treatment with 2000 rads did not hinder the function of these cells.

The results showing the necessity of a small percentage of adherent cells for the induction of suppressor cell activity raised

Figure 3. Effect of adherent cells on the induction suppressor cells by Con A. Comparison of the Con A induced suppressor cell activity of T cells cultured with adherent cells to those cultured without adherent cells.

EFFECT OF ADHERENT CELLS on the Induction of Suppressor Cells by Con A (9 Experiments)

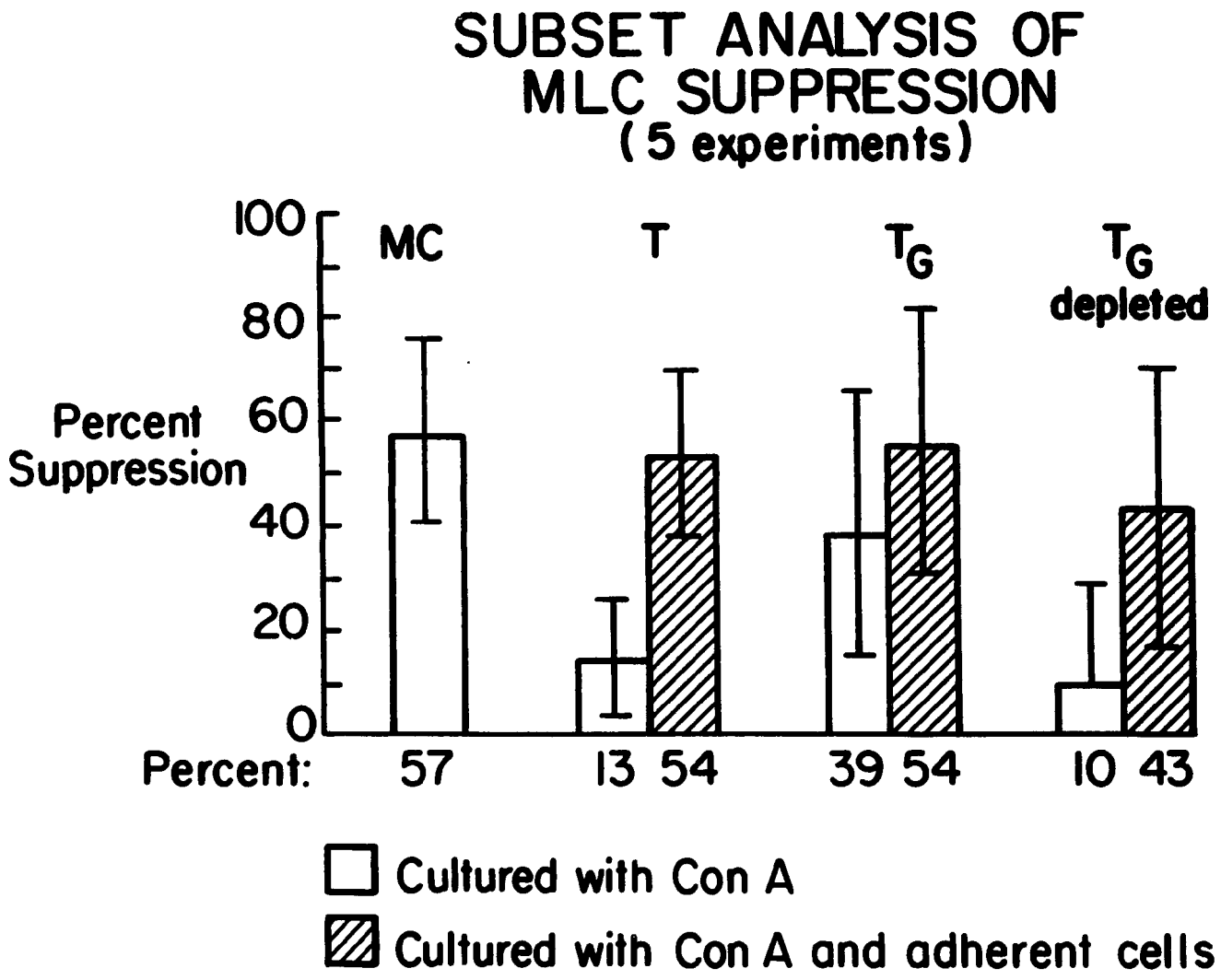


the possibility that the localization of suppressor cell activity in the T_G fraction in early experiments was artifactual. Isolated T_G cells consistently contained at least 1-2% monocytes, as determined by latex ingestion or peroxidase staining, whereas monocytes were undetectable in T_G depleted populations. The small monocyte contaminant in T_G fractions could be sufficient for inducing suppressor cell activity in this population while the absence of accessory cells in the T_G depleted fractions would not allow development of suppressor cell function. Consequently the effect of adherent cells on the T_G depleted fractions was examined. When 5% adherent cells were added to T_G depleted cells during Con A culture, these cells suppressed MLCs by an average of 43% while T_G cells suppressed an average of 54% (Figure 4). Thus the presence of adherent cells during Con A culture allowed both T_G and T_G depleted populations to develop suppressor cell activity although cells in the T_G fraction were more efficient.

The accepted theory at the time these studies were performed was the exclusivity of human suppressor cell function by cells expressing FcIgG receptors. These experiments demonstrating suppressor cell activity in T_G depleted populations were contradictory to this theory. Additional experiments were performed in an attempt to account for this discrepancy.

The possibility that Con A activation stimulated cells in the T_G depleted fraction to express FcIgG receptors was examined. If indeed the FcIgG receptor was a marker for suppressor cells this would explain the presence of suppressor cell activity in the T_G depleted fraction. It was found that after Con A activation 6-10%

Figure 4. Subset analysis of MLC suppression. The effect of adherent cells on the Con A induced suppressor cell activity of T cell subpopulations. Comparison of the suppressor cell activity of T_G and T_G deprived subpopulations cultured with adherent cells.


SUBSET ANALYSIS OF
MLC SUPPRESSION
(5 experiments)
Percent Suppression
100
80
60
40
20
0
MC
T
T_G
T_G depleted
Percent:
57
13 54
39 54
10 43
Cultured with Con A
Cultured with Con A and adherent cells

of the cells in the T_G depleted population were $FcIgG^+$. In light of this finding, the separation of cells PBMC into T cell subpopulations was performed after Con A culture. In these experiments both T_G and T_G deprived populations exhibited maximum suppressor cell activity.

In order to determine whether those T cells in the T_G deprived fraction which express neither FcIgG or FcIgM receptors (T null cells) were responsible for the suppressor cell activity in this fraction, the FcIgM receptor was utilized. T cells were separated into T_M and T_M deprived fractions. These populations were cultured with Con A and adherent cells and tested for suppressor cell activity. T_M and T_M depleted populations displayed equivalent suppressor cell function.

AMT/M4$^+$ T Cells

Since the investigator was unable to identify the Con A induced suppressor cell on the basis of FcIgG or FcIgM receptors, she turned to other putative suppressor cell markers. The second marker to be studied was the antigen recognized by the AMT/M4 antiserum developed by Balch (Ades et al., 1978). The AMT/M4 antiserum reacted with a 16,000 molecular weight antigen present on a subpopulation of T cells which exhibited suppressor cell activity in PWM induced B cell differentiation assays (Balch et al., 1981). ER^+ T cells were stained with fluoresceinated AMT/M4 and separated into AMT/M4 bright and AMT/M4 dull populations in a FACS. As a control, T cells were stained with AMT/M4 and not sorted. The

sorted subpopulations and the control unsorted cells were cultured with Con A and 5% adherent cells and then tested for suppressor cell activity in a MLR. AMT/M4 bright and dull cells suppressed an MLC by an average of 49% and 43% respectively (Figure 5). Thus the Con A suppressor cell could not be delineated on the basis of the 16,000 molecular weight antigen detected by AMT/M4.

DR^+ T Cells

The third marker to be tested was the DR antigen present on a subpopulation of activated human T cells (Ko et al., 1979; Reinherz et al., 1979b and 1979c). Only 1-2% of resting T cells expressed DR antigens but after activation with Con A it was found that 10-15% express this antigen. Since I region antigens are expressed on mouse suppressor T cells, it was reasonable to postulate that DR antigens might be expressed on human suppressor T cells. To test this hypothesis, Con A activated T cells were stained with mouse monoclonal anti-DR. The DR^+ cells were separated from DR^- cells by a rosetting technique utilizing SRBC coated with rabbit anti-mouse Ig. The DR^+ and DR^- cells were tested for suppressor cell activity in a MLR. The DR^+ cells suppressed an MLC by an average of 64% whereas DR^- cells suppressed by an average of 37% (Figure 6). Although there was some enrichment of suppressor cell function in the DR^+ cell fraction, all Con A induced suppressor cells did not express this antigen.

Figure 5. Subset analysis of MLC suppression. Comparison of the Con A induced suppression of MLCs by AMT/M4 bright and AMT/M4 dull T cell subpopulations.

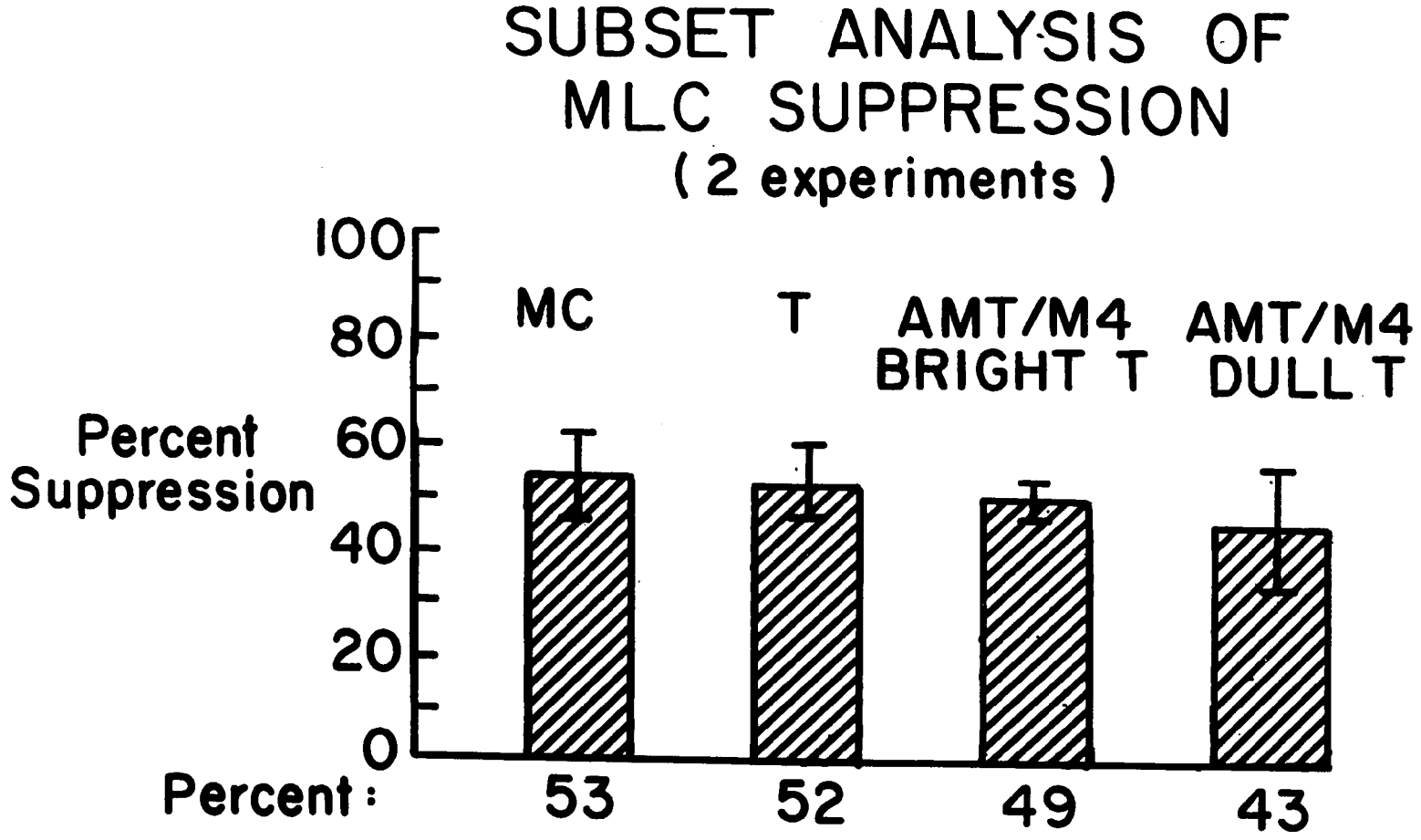
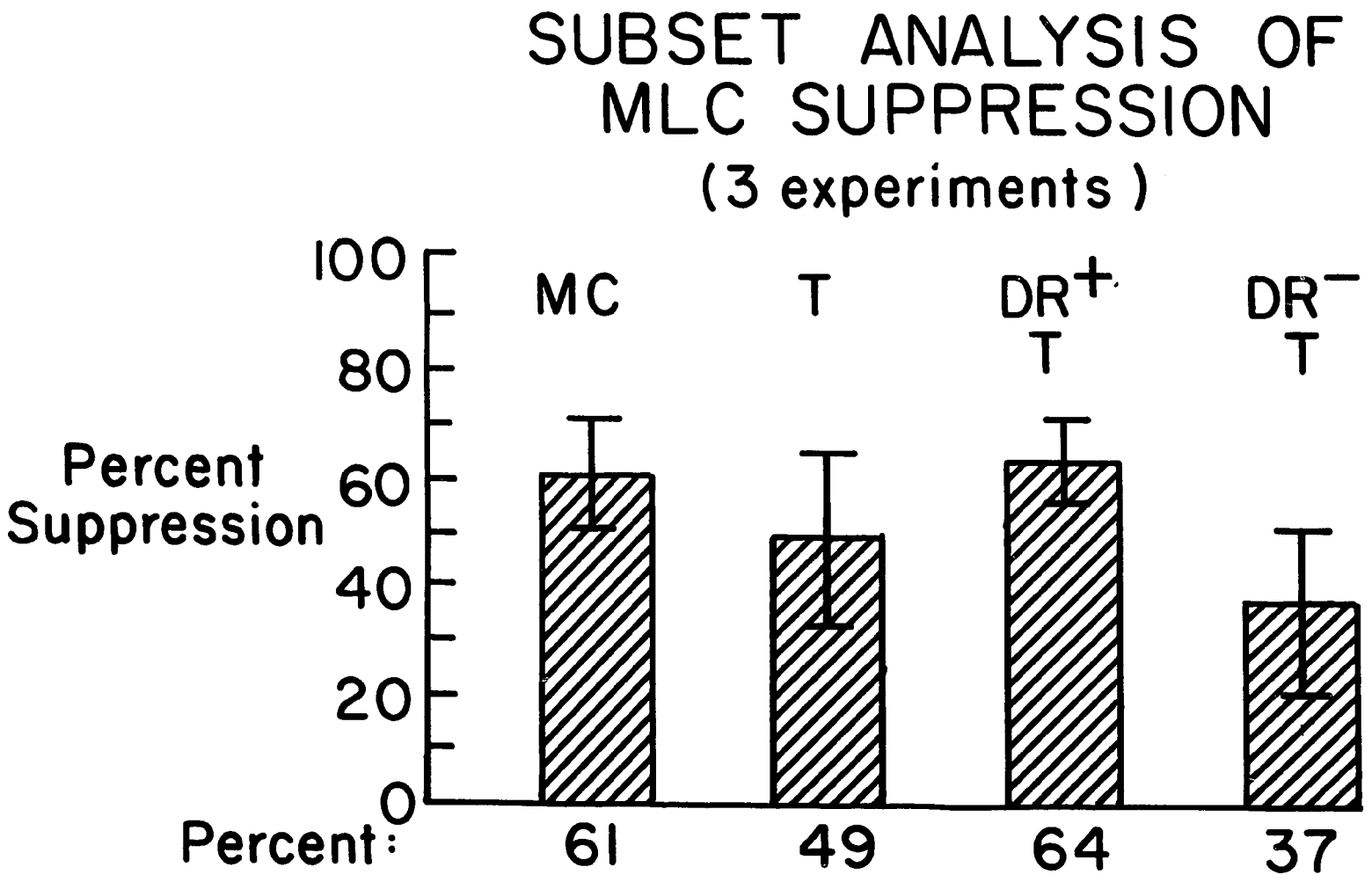

SUBSET ANALYSIS OF
MLC SUPPRESSION
(2 experiments)
Percent
Suppression
100
80
60
40
20
0
MC
T
AMT/M4
BRIGHT T
AMT/M4
DULL T
Percent :
53
52
49
43

Figure 6. Subset analysis of MLC suppression. Comparison of the Con A induced suppression of MLCs by DR^+ and DR^- activated T cells.


SUBSET ANALYSIS OF
MLC SUPPRESSION
(3 experiments)
Percent
Suppression
100
80
60
40
20
0
MC
T
DR+
T
DR−
T
Percent:
61
49
64
37

Discussion

The results of this study indicated that the induction of human suppressor cells by Con A requires an interaction between T cells and adherent cells. This finding was later reported by Raff and coworkers (1978) who showed that the adherent cell required was a low density macrophage bearing DR antigens. Such an interaction between macrophages and T cells in the induction of human suppressor cells is consistent with the known role of macrophages in the activation of murine T cells (Nelson, 1976).

One of the most important points to be made from these experiments is the necessity for pure populations of cells when attempting to define the functional characteristics of different populations. These experiments indicate that a level of impurity usually considered insignificant (1-2%) can make a critical difference in the results obtained. Early experiments in this study indicated a localization of Con A suppressor cells in the fraction expressing FcIgG receptors. Further investigation showed that these results were due to the small monocyte contaminant in the T_G fraction. In subsequent experiments, monocytes were added to the T_G deprived fraction during Con A culture and these cells also developed suppressor cell activity. Thus the Con A suppressor cell cannot be distinguished on the basis of the FcIgG receptor. These results corroborated those of Hayward and coworkers (1978) which showed that T_M cells stimulated with Con A would suppress PWM induced B cell differentiation. A conflicting report from

Herscowitz and colleagues (1980) states that only T_G cells functioned as precursors of Con A suppressor cells in a MLR whereas both $FcIgG^+$ and $FcIgG^-$ cells could be activated to suppress PHA induced proliferation and PWM induced polyclonal B cell immunoglobulin production. The data of these investigators showed an enrichment of suppressor cell activity in the T_G fraction and a depletion, not a total lack of suppressor cell activity, in the T_G deprived fraction. Thus their interpretation is contrary to this investigator while the data are similar. The author performed additional experiments when she found that after Con A stimulation some cells in the T_G deprived fraction expressed FcIgG receptors. In these experiments T_G and T_G deprived cells were separated after Con A stimulation. These two fractions displayed equivalent suppressor cell activity. Furthermore, T_M and T_M deprived fractions express comparable suppressor cell activity. This additional data lends support to the interpretation that both $FcIgG^+$ and $FcIgG^-$ cells can function as suppressor cells in a MLR.

Two additional T cell subsets were examined in an attempt to define the Con A suppressor cell by surface phenotype. The cells capable of Con A induced suppression of a MLC were not distinguished by the 16,000 molecular weight antigen detected by AMT/M4. The subset of T cells expressing this antigen were further characterized in our laboratory and although this subset functioned as suppressor cells in PWM induced B cell differentiation assays (Balch et al., 1981), these cells did not suppress a proliferative response to tetanus toxoid (unpublished observation). Thus this

marker distinguished a suppressor cell for B cell differentiation but not T cell proliferative responses. The subset of Con A activated T cells which express DR antigens was found to be a potent suppressor in a MLR. However, Con A suppressor cells were not confined to the DR^+ population since DR^- cells had significant suppressor cell activity.

Although the cell surface markers which the author used to characterize the Con A induced suppressor cell were not unique suppressor cell markers; the hypothesis that suppressor cells are a phenotypically distinct subset of T cells has since been proven by Reinherz and coworkers (1980). These investigators prepared a monoclonal antibody which delineates the human cytotoxic/suppressor T cell subset. The hybridoma technique for monoclonal antibodies has provided the definition of functional subsets of human T lymphocytes which was heretofore not possible. Thus, in addition to the OKT5 reagent specific for suppressor T cells, Reinherz and colleagues (1979a and 1979b) have prepared monoclonal reagents defining thymocytes, mature T cells and helper T cells. The nature of these monoclonal reagents allows a consistency and permanency in the definition of human T cells which will permit great advances in the understanding of the human immune response.

IMMUNOSUPPRESSION IN MELANOMA PATIENTS

Introduction

Decreased immunocompetence has been observed in patients with a variety of diseases. In the past these decreases in immune responses were thought to be due to absent or defective effector cells. In recent years it has been demonstrated that depressed immunity in many cases is related to abnormalities of immune regulation with imbalances of suppressor and helper cell function (Stobo et al., 1976; Moretta et al., 1977; Reinherz et al., 1980). Increased suppressor cell activity has been demostrated both in animals and humans with malignant tumors (Goodwin et al., 1977b; Broder et al., 1975; Yu et al., 1977).

A number of studies have shown that patients with malignant melanoma demonstrate depressed immunological responses. These defects include impaired responses in both primary and secondary delayed cutaneous hypersensitivity reactions (Golub et al., 1974; Eilber et al., 1975) and depressed proliferative responses to both mitogens and alloantigens (Golub et al., 1974; Zembala et al., 1977). The author proposed that the immunosuppression of these patients was due to a defect in immune regulation. To test this hypothesis she examined melanoma patients for aberrations in suppressor cell function.

A number of suppressor cell systems have been identified in man. These include suppressor monocytes in addition to the Con A induced suppressor T cell. Monocytes have been shown to inhibit lymphocyte responses by several mechanisms including the elaboration of prostaglandin (PG) (Goodwin et al., 1977a; Kurland and Bockman, 1978; Rice et al., 1979; Metzger et al., 1980).

The probable importance of PG-mediated suppression in tumor bearing animals has become evident in recent years. PG-mediated immune suppression has been found to influence several parameters of immune function in animals bearing both viral and chemically-induced tumors. For example, PG has been shown in tumor-bearing mice to inhibit natural killer (NK) activity (Droller et al., 1978b; Brunda et al., 1980) and to suppress the plaque-forming cell responses (Fulton and Levy, 1980). The administration of indomethacin, a PG synthetase inhibitor, in vitro and in vivo enhances both mitogen responses and NK activity in mice bearing either fibrosarcoma, mammary adenocarcinoma or Cloudman melanoma (Pelus and Strausser, 1976; Droller et al., 1978a; Brunda et al., 1980). Pelus and Bockman (1979) found that splenic macrophages from animals bearing methylcholanthrene-induced fibrosarcomas or Maloney sarcoma virus-induced tumors produced significantly more PG than macrophages from normal non-tumor bearing littermates.

Some animal tumors have been shown to elaborate high levels of PG relative to normal tissues (Plescia et al., 1975; Karim, 1976; Trevisani et al., 1980). Grinwich and Plescia (1977)

reported that PG produced by mouse fibrosarcoma cells suppressed antibody production in vitro and that indomethacin blocked this immunosuppression. They and others have also made the important observation that in vivo administration of indomethacin retarded growth of the chemically-induced fibrosarcoma tumors (Plescia et al., 1975; Grinwich and Plescia, 1977; Lynch and Salomon, 1979) and hepatoma tumors (Trevisani et al., 1980).

Another point to be made concerning the importance of PG-mediated suppression in tumor immunology is the influence it may have on the therapeutic effectiveness of immunotherapy. It has been assumed that immunotherapy acts by exerting a positive effect on lymphocyte and monocyte function. However, there is experimental evidence that BCG and C. parvum immunotherapy actually enhance PG-mediated suppression (Grimm et al., 1978; Savary and Lotzova, 1978; Ojo et al., 1978). Furthermore, recent studies indicate that indomethacin increases the therapeutic effectiveness of these immune stimulants in sarcoma bearing mice (Grimm et al., 1978; Lynch and Salomon, 1979; Tracy and Adkinson, 1980).

It is important to evaluate the effect of PG on different types of tumors. In one type, the mouse B-16 melanoma, an opposite relationship of PG and indomethacin has been demonstrated. Thus PG decreases the growth rate of this tumor in vivo while indomethacin enhances tumor growth (Favalli et al., 1980).

Abnormalities in PG-mediated suppression have been shown in humans with malignant tumors as well as in tumor bearing animals. Goodwin and colleagues (1977b) found that the depressed mitogen

responses of Hodgkin's Disease patients were partially restored in the presence of indomethacin. They found that the hyporesponsiveness to mitogens was due to excessive production of PGE_2 by PBMC from these patients.

In light of the information on immunosuppression in animals and humans with malignant tumors, the author performed a series of experiments to confirm the decreased immunocompetence of melanoma patients. The relationship of PG-mediated suppression to this decreased immunocompetence was examined.

Proposal

Hypothesis: The decreased immunocompetence of melanoma patients is due to imbalances in immune regulation.

Objective: To determine whether melanoma patients have an abnormality in immune regulation which results in decreased immunocompetence and to determine whether this abnormality is in help or suppression.

Rationale: Imbalances in immune regulation such as inadequate help or excess suppression have been demonstrated in many diseases in which patients exhibit decreased immunocompetence.

Results

Mitogen Response

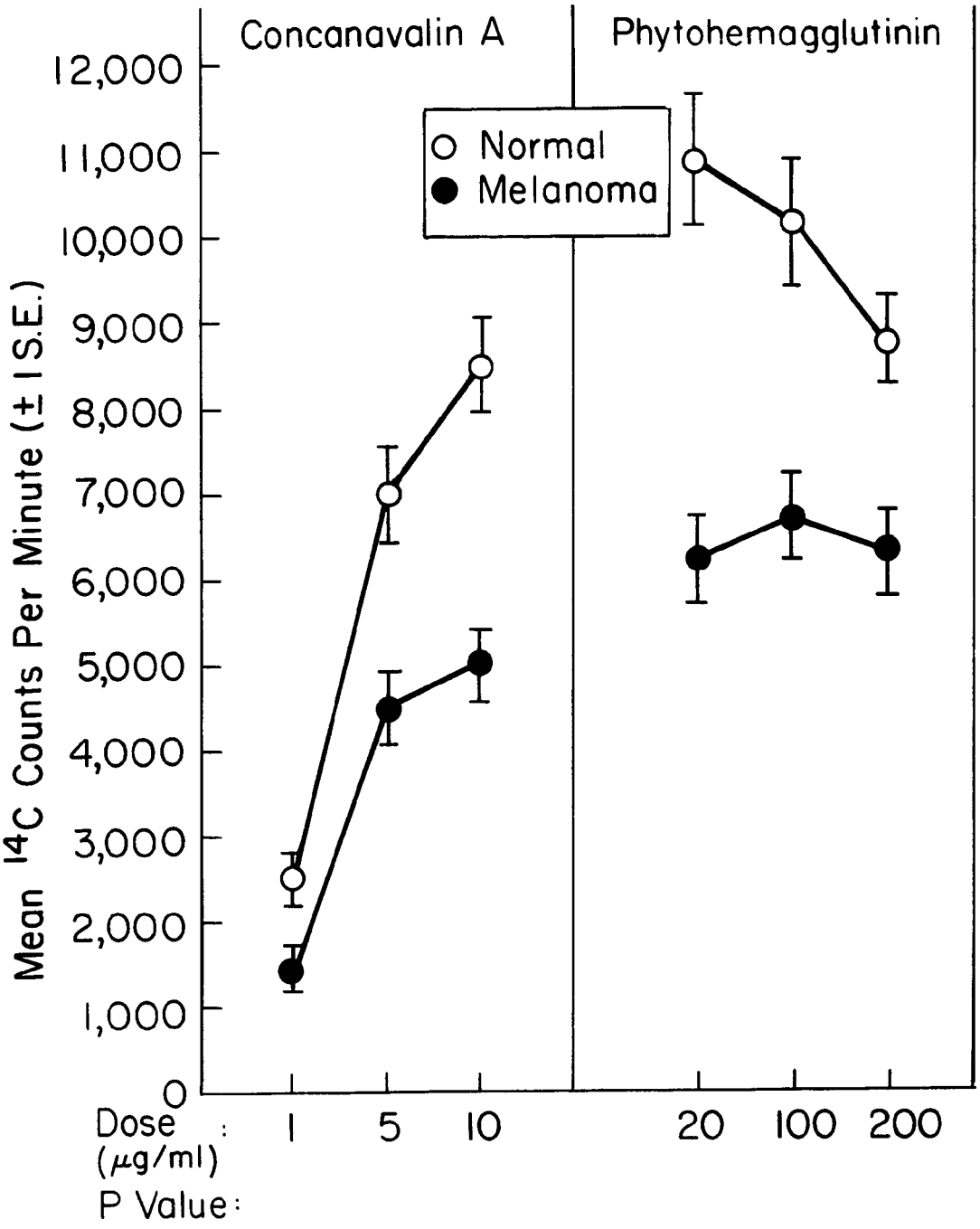
It is known that melanoma patients exhibit a variety of defects in cellular immunity. Decreased proliferative responses to different plant lectins, particularly Con A, are reproducible *in vitro* abnormalities that correlate well with *in vivo* measures of immunocompetence (Golub et al., 1974). Thus the mitogen response to Con A and PHA was used as a measure of immunocompetence in melanoma patients.

The proliferative responses to Con A and PHA were compared in 33 melanoma patients and 29 normal individuals. The magnitude of ^{14}C-thymidine incorporation by PBMC from melanoma patients was significantly depressed compared to normal control values for three concentrations of Con A and PHA ($p < 0.03$, Figure 7). In 32 out of 33 melanoma patients the response to one or both of the two mitogens was below the normal range.

Con A Suppressor Cells

The melanoma patients exhibited decreased immunocompetence as determined by subnormal responses to Con A and PHA. Experiments were performed to test the hypothesis that melanoma patients had excess Con A inducible suppressor cells which contributed to decreased mitogen responses. PBMC from melanoma patients were incubated with and without Con A for 48 hours. The control and Con A activated cells were tested for suppressor cell activity in MLCs

Figure 7. Comparison of the blastogenic response (^{14}C Thymidine incorporation) of 33 melanoma patients and 29 normal controls when their blood mononuclear cells were cultured with three doses of Con A or PHA.


Concanavalin A
Phytohemagglutinin
Normal
Melanoma
Mean ^{14}C Counts Per Minute (± 1 S.E.)
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
Dose (μg/ml)
1
5
10
20
100
200
P Value:

to determine whether melanoma patients exhibited abnormalities in this form of suppression. The patients tested did not deviate from normal individuals in Con A induced suppressor cell activity (Table I).

Effect of Indomethacin

In order to determine whether prostaglandin mediated suppression was involved in the depressed mitogen response of melanoma patients, the effect of indomethacin on mitogen response was examined. The mitogens responses of melanoma patients and normal controls were measured in the presence and absence of 1 μg/ml indomethacin. Indomethacin caused a significant increase in the mean proliferative response of PBMC from melanoma patients whereas it caused little or no change in the response of PBMC from normal subjects (Figure 8). The percent increase in mitogen response was most pronounced at the lowest mitogen doses. Goodwin and colleagues (1978) have previously shown that indomethacin enhancement of lymphocyte proliferation is higher with submitogenic doses of lectins. At a 1 μg/ml dose of Con A, the mean response of melanoma patients increased from 56% to 81% of the normal response, while at the lowest PHA dose (20 μg/ml) the response increased from 57% to 77% of normal when the PBMC were incubated with indomethacin. Figure 9 shows the individual results of 29 melanoma patients compared to the mean results of 29 normal controls. Thus when prostaglandin production was blocked with indomethacin in

TABLE I

COMPARISON OF THE SUPPRESSION OF MLCs BY CONTROL AND CON A CULTURED CELLS FROM MELANOMA PATIENTS AND NORMAL CONTROLS

	Control cultured cells	Con A cultured cells
Melanoma Patients (4)[a]	27 ± 4[b]	68 ± 7
Normal Controls (10)	21 ± 4	70 ± 5

(a) number of individuals studied

(b) mean % suppression ± 1 SE

Figure 8. The effect of indomethacin on mitogen responses in melanoma patients and normal individuals. The data show the mean percent increase of blastogenic responses to three doses of Con A and phytohemagglutinin by 33 melanoma patients and 29 normal subjects. The mitogen dose designated as dose #1 was either 1 μg/ml Con A or 20 μg/ml PHA, dose 2 was either 5 μg/ml Con A or 100 μg/ml PHA; while dose 3 was either 10 μg/ml Con A or 200 μg/ml PHA.

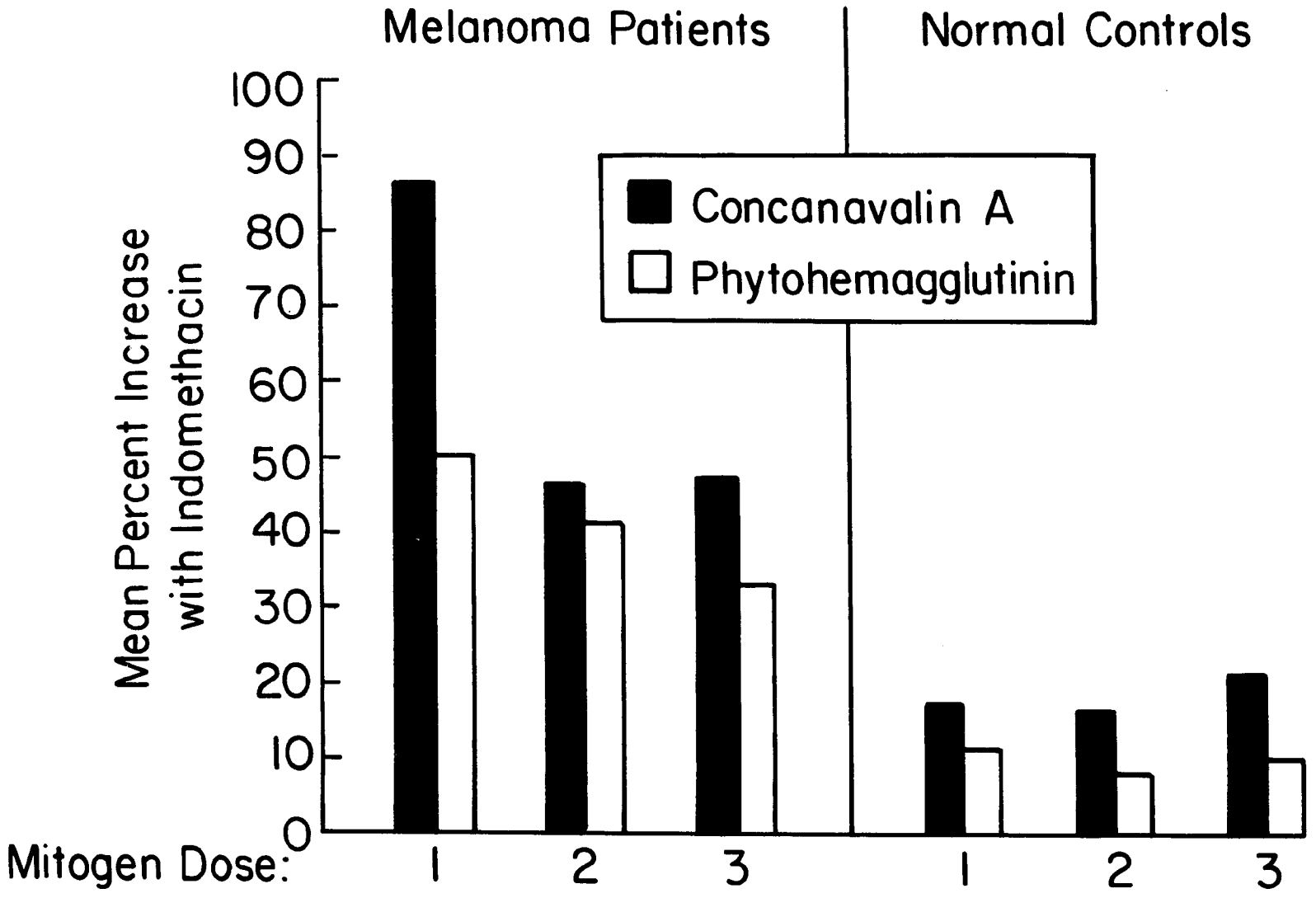
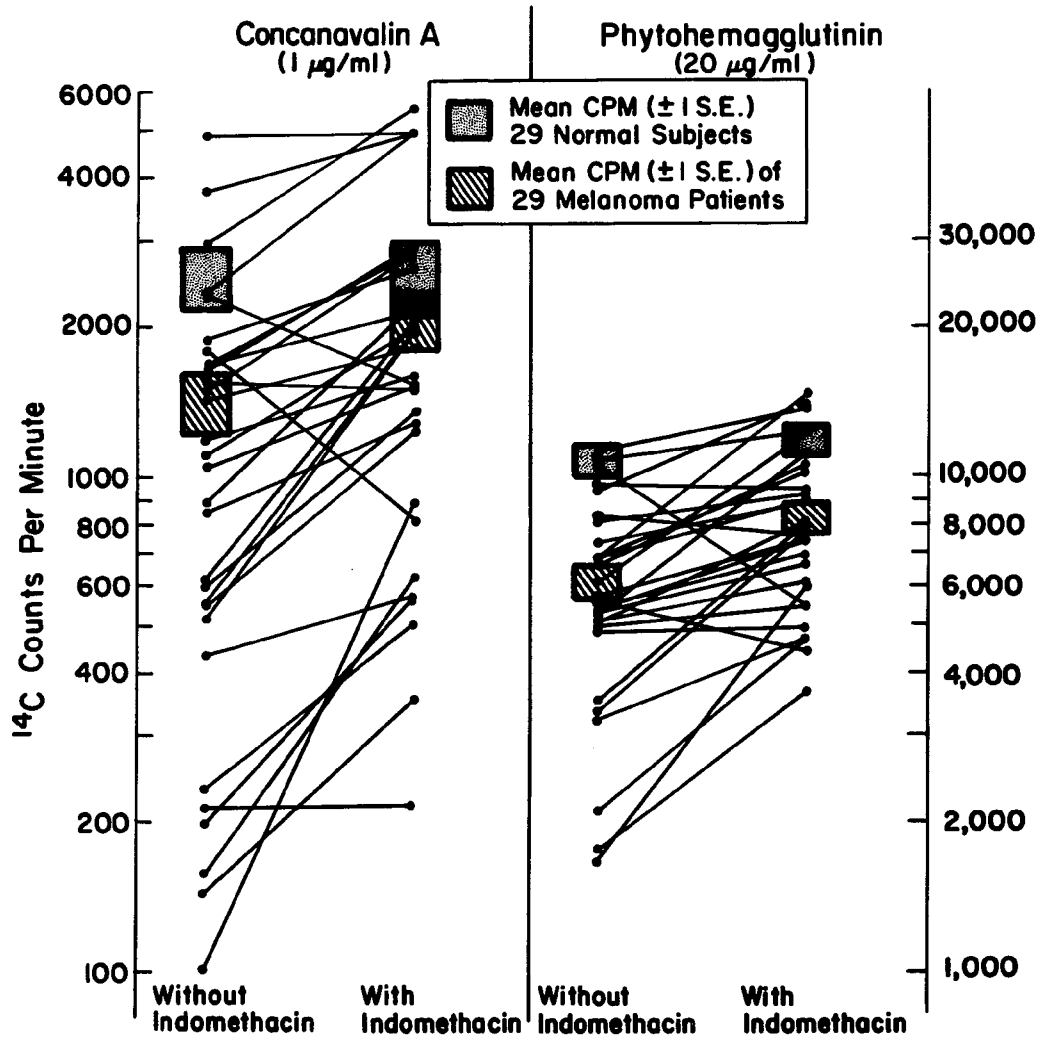

Melanoma Patients
Normal Controls
Concanavalin A
Phytohemagglutinin
Mean Percent Increase with Indomethacin
100
90
80
70
60
50
40
30
20
10
0
Mitogen Dose:
1
2
3
1
2
3

Figure 9. Individual results of 29 melanoma patients when their blood mononuclear cells were stimulated with Con A (1 μg/ml) or PHA (20 μg/ml) and the response compared with and without indomethacin in the cultures. The hatched boxes represent the mean (± 1SE) of all the melanoma the patient responses while the shaded boxes show the mean results for 29 matched normal subjects.


Concanavalin A
(1 μg/ml)
Phytohemagglutinin
(20 μg/ml)
Mean CPM (± 1 S.E.)
29 Normal Subjects
Mean CPM (± 1 S.E.) of
29 Melanoma Patients
^{14}C Counts Per Minute
6000
4000
2000
1000
800
600
400
200
100
30,000
20,000
10,000
8,000
6,000
4,000
2,000
1,000
Without Indomethacin
With Indomethacin
Without Indomethacin
With Indomethacin

these cultures there was a restoration of the depressed level of mitogen response in melanoma patients towards that of normal controls.

Removal of PGE Producing Cells

The monocyte has been shown to be the major prostaglandin producing cell among normal human PBMC (Goodwin et al., 1977a). Isolated ER^+ T cells were tested in the mitogen assay to determine whether the effect of indomethacin would be abrogated when monocytes were removed. In a sample of eight melanoma patients and four normal controls, indomethacin did not significantly enhance the PHA responses of ER^+ T cells for either the melanoma patients or the normal controls. In contrast, the PBMC response to PHA was enhanced by indomethacin for the melanoma patients but not for the normal subjects. Furthermore, removing the monocytes eliminated the differences in the PHA responses of melanoma patients and normal controls (Table II).

Stage of Disease and Age of the Patient

The melanoma patient data were subdivided by stage of disease to determine whether the degree of enhancement with indomethacin was related to the extent of tumor dissemination. At all doses of Con A and PHA, the level of ^{14}C-thymidine incorporation was lower for patients with regional and distant metastatic disease (Stage II and Stage III) compared to those with local disease (Stage I). Furthermore, the increase in mitogen response in the presence of

TABLE II

COMPARISON OF THE PHA RESPONSES OF ER^+ T CELLS FROM MELANOMA PATIENTS AND NORMAL CONTROLS IN THE PRESENCE AND ABSENCE OF INDOMETHACIN (1 μg/ml)

	Without Indomethacin	With Indomethacin
Normal (4)[a]	5128 ± 910[b]	5019 ± 714
Melanoma (8)	5140 ± 764	5593 ± 966

(a) Number of patients studied.

(b) Mean counts per minute ^{14}C thymidine ± one SE

indomethacin was greater in patients with metastatic disease than in those with localized disease (Table III). However, these differences were not statistically significant. The eight melanoma patients with metastases who were receiving immunotherapy did not have a significantly different response than those who had surgical treatment alone.

The age of the individual was also examined as a separate variable, since it has previously been shown that prostaglandin mediated suppression increases as a function of age (Goodwin and Messner, 1979). Although in both the control and patient groups there was a slight trend for the indomethacin effect to increase with age, the magnitude of increase within each age group was significantly greater for the melanoma patients (Figure 10). Another variable, the sex of the individual, also did not correlate with the degree of enhancement in the presence of indomethacin.

PGE Production

At this point in these studies it appeared that melanoma patients had an abnormality in prostaglandin mediated suppression since their mitogen response was enhanced with indomethacin. One possible mechanism for an aberration in this form of suppression is excessive production of PGE. The author adopted a RIA for measuring PGE_2 levels in culture in order to test this possibility.

PGE production by cultured PBC of melanoma patients was measured and compared to that of cultured PBMC of normal individuals. PBMC (5×10^5 cells/ml) were cultured for 48 hours,

TABLE III

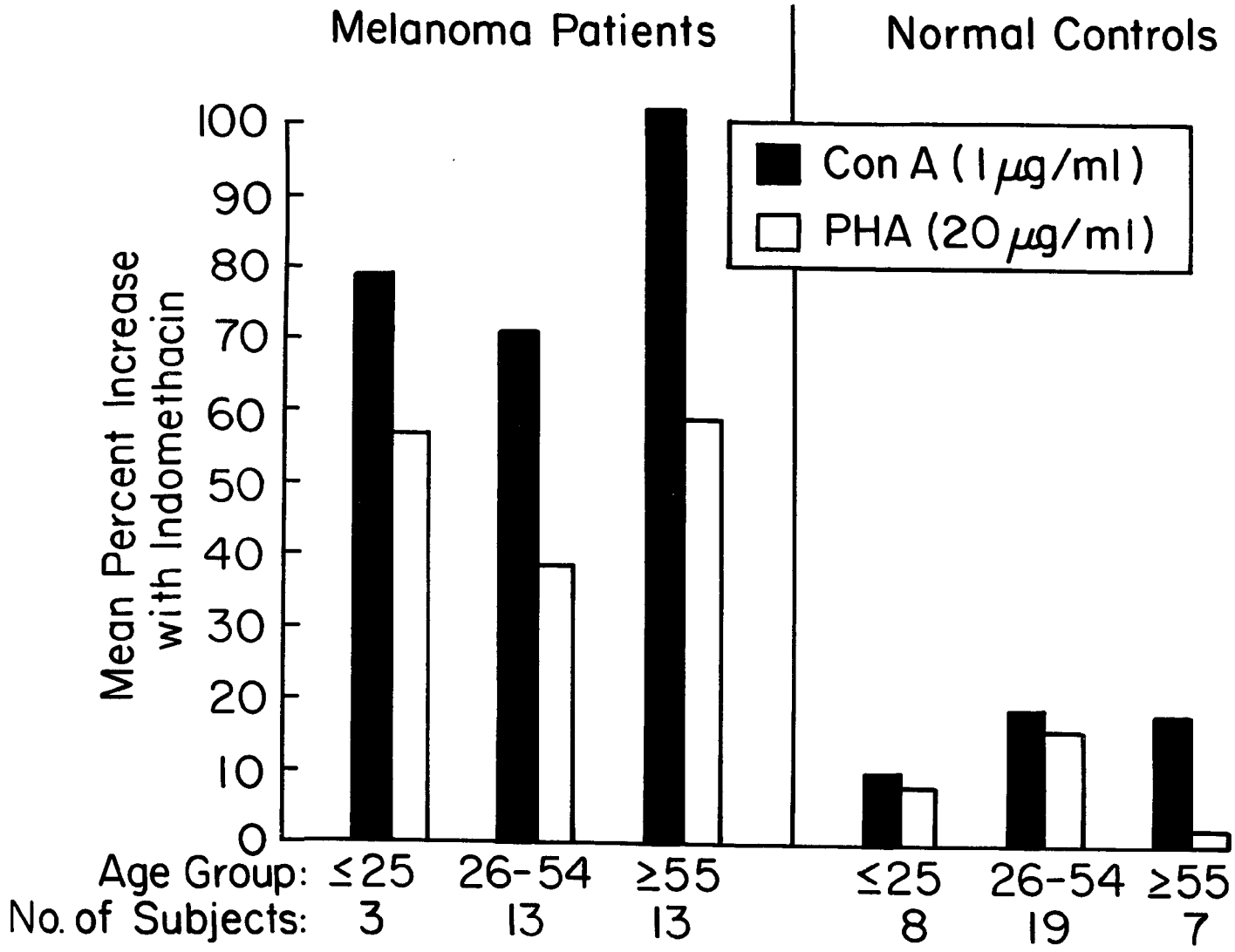
COMPARISON OF THE INDOMETHACIN EFFECT ON BLASTOGENIC RESPONSE OF LYMPHOCYTES FROM 21 MELANOMA PATIENTS WITH LOCALIZED DISEASE AND 12 PATIENTS WITH METASTATIC DISEASE

Mitogen[a]	Dose	Localized Melanoma (Stage I)	Metastatic Melanoma (Stage II and III)
Con A	1 µg/ml	81 ± 23 (18)[b]	95 ± 24 (11)
"	5 µg/ml	38 ± 14 (18)	58 ± 13 (11)
"	10 µg/ml	34 ± 11 (21)	69 ± 40 (11)
PHA	20 µg/ml	38 ± 12 (19)	69 ± 22 (12)
"	100 µg/ml	38 ± 11 (21)	46 ± 22 (12)
"	200 µg/ml	25 ± 9 (21)	47 ± 29 (12)

(a) Plus indomethacin (1 µg/ml) in all cultures.

(b) Number of patients tested in each group.

Figure 10. The effect of age on the level of indomethacin enhancement of mitogen response. Within each age group, the melanoma patients had a higher level of prostaglandin-mediated suppression compared to normal subjects. The data shows the mean percent increase of blastogenic response to Concanavalin A at (1 μg/ml) and phytohemagglutinin at (20 μg/ml) when cultured in the presence of indomethacin (1 μg/ml).


Melanoma Patients
Normal Controls
Con A (1 μg/ml)
PHA (20 μg/ml)
Mean Percent Increase with Indomethacin
100
90
80
70
60
50
40
30
20
10
0
Age Group: ≤25 26-54 ≥55 ≤25 26-54 ≥55
No. of Subjects: 3 13 13 8 19 7

supernatants harvested and then tested in a RIA for PGE_2. In a sample of 12 melanoma patients and 36 normal controls, the amount of PGE_2 produced by cultured PBMC was essentially the same for patients and controls (3.92 ± 0.69 ng/ml vs. 4.89 ± 0.49 ng/ml, respectively).

Sensitivity to Exogenous PGE_2

The monocytes of melanoma patients did not produce excess PGE; therefore, the possibility existed that the indomethacin effect was due to an increased sensitivity of melanoma patient lymphocytes to the inhibitory effects of PGE. The suppressive effects of exogenous PGE_2 were compared to PBMC of melanoma patients and of normal individuals. In these experiments the magnitude of Con A and PHA responses in the presence of a range of PGE_2 doses was measured in order to determine whether lymphocyte function in melanoma patients might be more easily suppressed by equivalent doses of PGE_2. Indomethacin was included in these cultures to block endogenous prostaglandin production. The results showed the PGE_2 dose response curves for PBMC of melanoma did not differ significantly from that obtained for normal controls (Table IV).

Effect of RO-205720

The results showing that melanoma patient PBMC did not produce excess PGE and were not hypersenstive to inhibition by PGE_2 suggested that the increase in their mitogen response in the presence of indomethacin was unrelated to PGE and might be due to

TABLE IV

COMPARISON OF THE SUPPRESSIVE EFFECTS OF EXOGENOUS PGE_2 ON THE CON A AND PHA RESPONSES OF PBMC FROM TEN MELANOMA PATIENTS AND SEVEN NORMAL CONTROLS

	Con A (1μg/ml)		PHA (20 μg/ml)	
PGE_2 Dose (Moles)	Melanoma Patients	Normal Controls	Melanoma Patients	Normal Controls
3×10^{-9}	19 ± 7[(a)]	26 ± 5	11 ± 6	18 ± 4
3×10^{-8}	39 ± 5	46 ± 3	42 ± 6	43 ± 5
3×10^{-7}	52 ± 4	57 ± 2	57 ± 5	60 ± 4
3×10^{-6}	59 ± 4	65 ± 3	65 ± 5	65 ± 4

(a) % Inhibition of the mitogen response ± one SE.

another of the reported effects of this drug (Shen and Winter, 1977; Weiss and Hart, 1977; Kantor and Hampton, 1978). RO-205720 is an experimental drug (Hoffman-La Roche) that is structurally unrelated to indomethacin and whose only known function is inhibition of prostaglandin synthesis. If the effect of indomethacin was due to inhibition of Prostaglandin synthesis and not some other action of the drug, then RO-205720 would mimic the effect of indomethacin on the mitogen responses of melanoma patients. In a sample of 13 melanoma patients, RO-205720 increased the PHA response by only 24 ± 10%, whereas indomethacin increased this response by 83 ± 19% ($p < .001$ by paired student t test). The mean percent change in the Con A response was -8 ± 8 with RO-205720 and 58 ± 12 ($p < .001$) with indomethacin. These results, in conjunction with the data on PGE production and the sensitivity to inhibition by PGE_2, indicate that the depressed mitogen response in melanoma patients is not related to abnormalities in prostaglandin mediated suppression.

Discussion

These experiments were designed to determine whether melanoma patients have an abnormality in immune regulation which results in decreased immunocompetence. The mitogen responses of PBMC of melanoma patients and normal individuals were determined in order to confirm a decrease in immune response in melanoma patients. As reported by other investigators (Golub et al., 1974; Eilber et al., 1975; Zembala et al., 1977); melanoma patients exhibited significantly depressed mitogen responses to Con A and PHA. The addition of indomethacin to mitogen cultures partially restored these responses whereas it had little effect on the responses of PBMC of normal individuals. The effect of indomethacin was not related to the melanoma patients: age, stage of disease, sex, or treatment.

The major pharmacological effect of indomethacin is the inhibition of PG synthesis by monocytes. However, it is known that indomethacin has direct effects on cellular function such as: (1) uncoupling of oxidative phosphorylation, (2) stabilization of lyzosomes, (3) membrane stabilization, and (4) inhibition of a number of enzymes (Shen and Winter, 1977). Two reported effects that may be important in terms of cellular proliferation are the perturbation of cyclic AMP levels through inhibition of phosphodiesterase (Weiss and Hart, 1977) and the inhibition of cyclic AMP dependent protein kinases (Kantor and Hampton, 1978). Although it is generally assumed that indomethacin effects are the result of PG synthetase inhibition, it is important to distinguish this effect from the non-PG related effects of indomethacin.

The defect in the PBMC of melanoma patients appeared to be due to an abnormality in monocyte function since the PHA responses of ER^+ T cells from normal individuals and melanoma patients were equivalent. Furthermore, the effect of indomethacin was on the monocyte because the response of ER^+ T cells was not increased in its presence. Thus the in vitro defect in immune competence for these melanoma patients appeared to be related to an abnormality in regulatory monocyte function. There are at least two explanations for this observed defect. First, melanoma patients might have an abnormality in PG-mediated suppression that was blocked by indomethacin. Alternatively, they might have a defect in monocyte function that was independent of PG metabolism. Three groups of experiments were performed to distinguish between these two possibilities.

In the first group of experiments the amount of PGE_2 produced by cultured PBMC of melanoma patients and normal individuals was compared. Goodwin and coworkers (1977b) had shown that excessive PGE_2 production by monocytes was partially responsible for the depressed mitogen response of Hodgkin's disease patients and our laboratory (Balch et al., 1981) had shown a similar defect in head and neck cancer patients. The melanoma patients did not demonstrate an increased level of PGE_2 production. Since melanoma patient PBMC produced normal amounts of PGE_2, a second group of experiments were performed to test the possibility that the lymphocytes of melanoma patients was hypersensitive to the inhibitory effects of PGE_2. The depressed mitogen responses of

elderly individuals had been reported to be due to an increased sensitivity to inhibition by PGE_2 (Goodwin and Messner, 1979). The magnitude of suppression of PBMC and ER^+ T cell mitogen responses by a range of PGE_2 concentrations was the same for melanoma patients and normal individuals. The results showing that melanoma patients did not exhibit excess production of PGE_2 or hypersensitivity to PGE_2 suggested that the effect of indomethacin was not related to PG-mediated suppression. To verify this conclusion a third group of experiments was performed in which the effect of another inhibitor of PG synthesis, RO-205720, was tested. In simultaneous mitogen assays, RO-205720 did not increase the response of melanoma patient PBMC while indomethacin did increase these responses. Thus the effects of indomethacin were not related to PG-mediated suppression.

These studies have indicated a defect in mitogen responsiveness in PBMC of melanoma patients which is partially corrected in the presence of indomethacin. Although it is generally assumed that indomethacin effects are the result of PG synthetase inhibition, it is important to distinguish that effect from the other pharmacological effects of indomethacin. This study indicates that the indomethacin enhancement of mitogen response in melanoma patients is not related to PG-mediated suppression. Indirect evidence implies that the target of the indomethacin effect is the monocyte. Continued experimentation will allow a more precise definition of the defect in melanoma patients.

BIBLIOGRAPHY

1. Ades, E.W., A. Bukacek, R.K. Zwerner, P.A. Dougherty, and C.M. Balch, 1978. Expression of two differentiation antigens on normal and cultured human T cells. J. Immunol. 121:513-519.

2. Balch, C.M., P.A. Dougherty, M.K. Dagg, A.G. Diethelm, and A.R. Lawton, 1977. Detection of human T cells using anti-monkey thymocyte antisera: Tissue distribution and evidence for antigenic heterogeneity. Clin. Immunol. Immunopath. 8:448-453.

3. Balch, C.M., P.A. Dougherty, and A.B. Tilden, 1981. Excessive prostaglandin E_2 production by suppressor cells in head and neck cancer patients. Proceedings ASCO/AACR (in press).

4. Balch, C.M., M.R. Loker, P.A. Dougherty, and E.W. Ades, 1981. Expression of a 16,000 molecular weight antigen on human suppressor T lymphocytes. Manuscript in preparation.

5. Bean, M.A., Y. Kodera, K.B. Cummings, and B.R. Bloom, 1977. Occurrence of restricted suppressor T-cell activity in man. J. Exp. Med. 146:1455-1464.

6. Bona, C., F. Audibert, D. Juy, and L. Chedid, 1976. Cell suppression in PPD-induced blast specific response of human peripheral blood lymphocytes. Clin. Exp. Immunol. 26:258-264.

7. Broder, S., R. Humphrey, M. Durm, M. Blackman, B. Meade, C. Goldman, W. Strober, and T. Waldmann, 1975. Impaired synthesis of polyclonal (non-paraprotein) immunoglobulins by circulating lymphocytes from patients with multiple myeloma - role of suppressor cells. N. Engl. J. Med. 293:887-890.

8. Broder, S., D. Poplack, J. Whang-Peng, et al., 1978. Characterization of a suppressor-cell leukemia: Evidence for the requirement of an interaction of two T cells in the development of human suppressor effector cells. N. Engl. J. Med. 298:66-70.

9. Broder, S. and T.A. Waldmann, 1978. The suppressor-cell network in cancer. New Engl. J. Med. 299:1281-1284.

10. Bukacek, A., M. Roper and C.M. Balch, 1979. A subpopulation of Human T cells bearing Fc receptors for IgG can be induced by Con A to suppress the mixed lymphocyte reaction (MLR). Fed. Proc. 38:1161.

11. Brunda, M.J., R.B. Herberman, and H.T. Holden, 1980. Inhibition of murine natural killer activity by prostaglandins. J. Immunol. 124:2682-2689.

12. Cantor, H., and E.A. Boyse, 1975a. Functional subclasses of T lymphocytes bearing different ly antigens: I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. J. Exp. Med. 141:1376-1389.

13. Cantor, H., and E.A. Boyse, 1975b. Functional subclasses of T lymphocytes bearing different ly antigens: II. Cooperation between subclasses of ly cells in the generation of killer activity. J. Exp. Med. 141:1390-1401.

14. Cantor, H., F.W. Shen, and E.A. Boyse, 1976. Separation of helper T cells from suppressor T cells expressing different ly components. II. Activation by antigen: After immunization, antigen-specific suppressor and helper activities are mediated by distinct T-cell subclasses. J. Exp. Med. 143:1391-1401.

15. Droller, M.J., P. Perlmann, and M.U. Schneider, 1978a. Enhancement of natural and antibody-dependent lymphocyte cytotoxicity by drugs which inhibit prostaglandin production by tumor target cells. Cell. Immunol. 39:154-163.

16. Droller, M.J., P. Perlmann, and M.U. Schneider, 1978b. A possible role of prostaglandins in the inhibition of natural and antibody-dependent cell-mediated cytotoxicity against tumor cells. Cell. Immunol. 39:165-177.

17. Eilber, F.R., J.A. Nizze, and D.L. Morton, 1975. Sequential evaluation of general immune competence in cancer patients. Correlation with clinical course. Cancer 35:660-664.

18. Engleman, E.G., R. Hoppe, H. Kaplan, et al., 1978. Suppressor cells of the mixed lymphocyte reaction in healthy subjects and patients with Hodgkin's disease and sarcoidosis. Clin. Res. 26:513-517.

19. Evans, R.L., J.M. Breard, A. Lazarus, S.F. Schlossman, and Chess, 1977. Detection isolation and functional characterization of two human T-cell subclasses bearing unique differentiation antigens. J. Exp. Med. 145:221-230.

20. Favalli, C., E. Garaci, E. Etheredge, M.G. Santoro, and B.M. Joffe, 1980. Influence of PGE on the immune response in melanoma-bearing mice. J. Immunol. 125:897-902.

21. Feldman, M., P.C.L. Beverly, J. Woody, and I.F.C. McKenzie, 1977. T-T interactions in the induction of suppressor and helper T cells: Analysis of membrane phenotype of precursor and amplifier cells. J. Exp. Med. 145:793-801.

22. Fujimoto, S., M. Greene, and A.H. Sehon, 1976a. Regulation of the immune response to tumor antigens. I. Immunosuppressor cells in tumor-bearing hosts. J. Immunol. 116:791-799.

23. Fujimoto, S., M. Greene and A.H. Sehon, 1976b. Regulation of the immune response to tumor antigens. II. The nature of immunosuppressor cells in tumor-bearing hosts. J. Immunol. 116:800-808.

24. Fulton, A.M., and J.G. Levy, 1980. The possible role of prostaglandins in mediating immune suppression by non-specific T suppressor cells. Cell. Immunol. 52:29-37.

25. Gershon, R.K. and K. Kondo, 1970. Cell interactions in the induction of tolerance: The role of thymic lymphocytes. Immunol. 18:723-734.

26. Gershon, R.K., and K. Kondo, 1971. Infectious immunologic tolerance. Immunol. 21:903-914.

27. Gold, E.R., and H.H. Fudenberg, 1967. Chromic chloride: A coupling reagent for passive hemagglutination reactions. J. Immunol. 99:859-866.

28. Golub, S.H., T.X. O'Connell, and D.L. Morton, 1974. Correlation of in vivo and in vitro assays of immunocompetence in cancer patients. Cancer Res. 34:1833-1836.

29. Goodwin, J.S., and R.P. Messner, 1979. Sensitivity of lymphocytes to prostaglandin E_2 increases in subjects over age 70. J. Clin. Invest. 64:434-439.

30. Goodwin, J.S., and D.R. Webb, 1980. Regulation of the immune response by prostaglandins. Clin. Immunol. Immunopath. 15:106-125.

31. Goodwin, J.S., A.D. Bankhurst, and R.P. Messner, 1977a. Suppression of human T-cell mitogenesis by prostagladin: Existence of a prostaglandin-producing suppressor cell. J. Exp. Med. 146:1719-1733.

32. Goodwin, J.S., R.P. Messner, A.D. Bankhurst, G.T. Peake, J.H. Saiki and R.C. Williams, Jr., 1977b. Prostaglandin-producing suppressor cells in Hodgkin's disease. N. Engl. J. Med. 297:963-967.

33. Goodwin, J.S., R.P. Messner, and G.T. Peake, 1978. Prostaglandin suppression of mitogen-stimulated lymphocytes. In vitro changes with mitogen dose and preincubation. J. Clin. Invest. 62:753-760.

34. Greene, M.E., M.E. Dorf, M. Pierres and B. Benacerraff, 1977. Reduction of syngeneic tumor growth by an anti-I-J alloantiserum. Proc. Natl. Acad Sci. U.S.A. 74:5118-5120.

35. Grimm, W., M. Seitz, H. Kirchner, and D. Gemsa, 1978. Prostaglandin synthesis in spleen cell cultures of mice injected with Corynebacterium parvum. Cell. Immunol. 40:419-426.

36. Grinwich, K.D., and O.J. Plescia, 1977. Tumor-mediated immunosuppressions. Prevention by inhibitors of prostaglandin synthesis. Prostaglandins 14:1175-1181.

37. Han, T., and H. Takita, 1980. Indomethacin-mediated enhancement of lymphocyte response to mitogens in healthy subjects and lung cancer patients. Cancer 46:2416-2422.

38. Haynes, B.F., and A.S. Fauci, 1977. Activation of human B lymphocytes. III. Concanavalin A induced generation of suppressor cells of the plaque-forming cell response of normal human B lymphocytes. J. Immunol. 118:2281-2289.

39. Hayward, A.R., L. Layward, P.M. Lydyard, L. Moretta, M. Dagg, and A.R. Lawton, 1978. Fc-receptor heterogeneity of human suppressor T cells. J. Immunol. 121:1-5.

40. Herscowitz, H.B., T. Sakane, A.D. Steinberg, and I. Green, 1980. Heterogeneity of human suppressor cells induced by Concanavalin A as determined in simultaneous assays of immune function. J. Immunol. 124:1403-1410.

41. Herzenberg, L.A., R.G. Sweet, and L.A. Herzenberg, 1976. Fluorescence activated cell sorting. Sci. Am. 234:108-117.

42. Horowitz, S., W. Borcherding, A.V. Moorthy, R. Chesney, H. Schulte-Wisserman, and R. Hong, 1977. Induction of suppressor T cells in systemic lupus erythematosus by thymosin and cultured thymic epithelium. Science 197:999-1001.

43. Hubert, C., G. Delespesse, and A. Govaerts, 1976. Concanavalin A-activated suppressor cells in normal human peripheral blood lymphocytes. Clin. Exp. Immunol. 26:95-98.

44. Indiveri, F., B.S. Wilson, M.A. Pellegrino, and S. Ferrone, 1979. Detection of human histocompatibility antigens with an indirect rosette microassay. J. Immunol. Methods 29:101-106.

45. Indiveri, F., B.S. Wilson, C. Russo, V. Quaranta, M.A. Pellegrino, and S. Ferrone, 1980. Ia-like antigens on human T lymphocytes: relationship to other surface markers, role in mixed lymphocyte reactions, and structural profile. J. Immunol. 125:2673-2678.

46. Jaffe, B.M., H.R. Behrman, and C.W. Parker, 1973. Radioimmunoassay measurement of prostaglandin E, A, and F in human plasma. J. Clin. Invest. 52:398-405.

47. Jerrells, T.R., J.H. Dean, G.L. Richardson, J.L. McCoy, and R.B. Herberman, 1978. Role of suppressor cells in depression of in vitro lymphoproliferative responses of lung and breast cancer patients. J. Natl. Cancer Inst. 61:1001-1006.

48. Jondal, M., G. Holm, and H. Wigzell, 1972. Surface markers on human T & B lymphocytes I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J. Exp. Med. 136:207-212.

49. Kantor, H.S., and M. Hampton, 1978. Indomethacin in submicromolar concentrations inhibit cyclic AMP-dependent protein kinase. Nature 276:841-842.

50. Karim, S.M.M., 1976. Prostaglandins and tumors. Adv. Prostaglandin Res. 2:303-320.

51. Kirchner, H., M. Glaser, and R.B. Herberman, 1975. Suppression of cell mediated tumor immunity by Corynebacterium parvum. Nature 257:396-398.

52. Ko, H.S., S.M. Fu, R.J. Winchester, D.T.Y. Yu, and H.G. Kunkel, 1979. Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen and antigen activated T cells. J. Exp. Med. 150:246-255.

53. Kurland, J.L., and R. Bockman, 1978. Prostaglandin E production by human monocytes and mouse macrophages. J. Exp. Med. 147:95-106.

54. Lum, L.G., A.V. Muchmore, D. Keren, J. Decker, I. Koski, W. Strober, and R.M. Blaese, 1979. A receptor for IgA on human T lymphocytes. J. Immunol. 122:65-73.

55. Lynch, N.R., and J.-C. Salomon, 1979. Tumor growth inhibition and potentiation of immunotherapy by indomethacin in mice. J. Natl. Cancer Inst. 62:117-121.

56. Metzger, Z., J.R. Hoffeld, and J.J. Oppenheim, 1980. Macrophage mediated suppression: I. Evidence for participation of both hydrogen peroxide and prostaglandins in suppression of murine lymphocyte proliferation. J. Immunol. 124:983-988.

57. Moretta, L., M. Ferrarini, M.C. Mingari, A. Moretta, and S.R. Webb, 1976. Subpopulations of human T cells identified by receptors for immunoglobulins and mitogen responsiveness. J. Immunol. 117:2171-2174.

58. Moretta, L., M.C. Mingari, S.R. Webb, E.R. Pearl, P.M. Lydyard, C.E. Grossi, A.R. Lawton, and M.D. Cooper, 1977. Imbalances in T cell subpopulations associated with immunodeficiency and autoimmune syndromes. Eur. J. Immunol. 7:696-702.

59. Moretta, L., S.R. Webb, C.E. Grossi, P.M. Lydyard, and M.D. Cooper, 1977. Functional analysis of two human T cell subpopulations; help and suppression of B-cell responses by T cells bearing receptors for IgM (T_M) or IgG (T_G). J. Exp. Med. 146:184-200.

60. Murphy, D.B., 1978. The I-J subregion of the murine H-2 gene complex. Semin. Immunopathol. 1:111-131.

61. Nelson, D.S. (ed.), 1976. Immunobiology of the Macrophage. Academic Press, New York.

62. Ojo, E., O. Haller, A. Kimura, and H. Wigzell, 1978. An analysis of conditions allowing Corynebacterium parvum to cause either augmentation or inhibition of natural killer cell activity against tumor cells in mice. Int. J. Cancer 21:444-452.

63. Owen, F.L., and M.W. Fanger, 1976. Studies on the human T-lymphocyte population -IV. The isolation of T-lymphocyte antigens from peripheral lymphocytes. Immunochemistry 13:121-132.

64. Pellegrino, M.A., S. Ferrone, M.P. Dierich, and R.A. Reisfeld, 1975. Enhancement of sheep red blood cell human lymphocyte rosette formation by the sulfhydryl compound 2-amino ethylisothiouronium bromide. Clin. Immunol. Immunopath. 3:324-333.

65. Pelus, L.M., and H.R. Strausser, 1976. Indomethacin enhancement of spleen-cell responsiveness to mitogen stimulation in tumorous mice. Int. J. Cancer 18:653-660.

66. Pelus, L. and R. Bockman, 1979. Increased prostaglandin synthesis by macrophages from tumor-bearing mice. J. Immunol. 123:2118.

67. Plescia, O.J., A.H. Smith, and K. Grinwich, 1975. Subversion of immune system by tumor cells and role of prostaglandins. Proc. Natl. Acad. Sci. 72:1848-1851.

68. Pierres, M., R.N. Germain, M.E. Dorf, and B. Benacerraf, 1978. In vivo effects of anti-Ia alloantisera. I. Elimination of specific suppression by in vivo administration of antisera specific for I-J controlled determinants. J. Exp. Med. 147: 656.

69. Raff, H.V., K.C. Cochrum, and J.D. Stobo, 1978. Macrophage-T cell interactions in the Con A induction of human suppressive T cells. J. Immunol. 121:2311-2315.

70. Reinherz, E.L., P.C. Kung, G. Goldstein, and S. F. Schlossman, 1979a. A monoclonal antibody with selective reactivity with functionally mature thymocytes and all peripheral human T cells. J. Immunol. 123:1312-1317.

71. Reinherz, E.L., P.C. Kung, J. M. Pesando, J. Ritz, G. Goldstein, and S.F. Schlossman, 1979b. Ia determinants on human T cell subsets defined by monoclonal. Activiation stimuli required for expression. J. Exp. Med. 150:1472-1482.

72. Reinherz, E.L., P.C. Kung, G. Goldstein, and S.F. Schlossman, 1979c. Further characterization of the human induced T cell subset defined by monoclonal antibody. J. Immunol. 123: 2894-2896.

73. Reinherz, E.L., P.C. Kung, G. Goldstein, and S.F. Schlossman, 1980. A monoclonal antibody reactive with the human cytotoxic/suppressor T cell subset previously defined by a heteroantiserum term TH_2. J. Immunol. 124:1301-1307.

74. Reinherz, E.L., and S.F. Schlossman, 1980. The differentiation and function of human T lymphocytes. Cell 19:821-827.

75. Rice, L., A.H. Laughter, and J.J. Twomey, 1979. Three suppressor systems in human blood that modulate lymphoproliferation. J. Immunol. 122:991-996.

76. Sakane, T., and I. Greene, 1977. Human suppressor T cells induced by concanavalin A: Suppressor T cells belong to distinctive T-cell subclasses. J. Immunol. 119:1169-1178.

77. Savary, C.A., and E. Lotzova, 1978. Suppression of natural killer cell cytotoxicity by splenocytes from Corynebacterium parvum-injected, bone marrow-tolerant, and infant mice. J. Immunol. 120:239-243.

78. Shen, T-Y., and C.A. Winter, 1977. Chemical and biological studies on indomethacin, sulindac and their analogs. Adv. Drug Res. 12:89-250.

79. Shou, L., S.A. Schwartz, and R.A. Good, 1976. Suppressor cell activity after concanavalin A treatment of lymphocytes from normal donors. J. Exp. Med. 143:1100-1110.

80. Stevens, R.H., and A. Saxon, 1978. Anti-human helper T-lymphocyte antiserum: generation and functional characterization. Clin. Immunol. Immunopath. 10:438-443.

81. Stobo, J.D., S. Paul, R.E. Van Scoy, and P.E. Hermans, 1976. Suppressor thymus-derived lymphocytes in fungal infection. J. Clin. Invest. 57:319-324.

82. Taffet, S.M., and S.W. Russell, 1981. Macrophage mediated tumor cell killing: Regulation of expression of cytolytic activity by Prostaglandin E. J. Immunol. 126:424-427.

83. Tilden, A.B., and C.M. Balch, 1981a. Indomethacin enhancement of immunocompetence in melanoma patients. Surgery (in press).

84. Tilden, A.B., and C.M. Balch, 1981b. Modulation of mitogen response by indomethacin in melanoma patients is not related to Prostaglandin E_2. Fed. Proc. 40:1088.

85. Tracy, D.E., and N.F. Adkinson, Jr., 1980. Prostaglandin synthesis inhibitors potentiate the BCG-induced augmentation of natural killer cell activity. J. Immunol. 125:136-141.

86. Trevisani, A., E. Ferretti, A. Capuzzo, and V. Tomasi, 1980. Elevated levels of prostaglandin E_2 in Yoshida hepatoma and the inhibition of tumor growth by non-steroidal anti-inflammatory drugs. Br. J. Cancer 41:341-346.

87. Tse, H.Y., and R.W. Dutton, 1977. Separation of helper and suppressor T lymphocytes. II. Ly phenotypes and lack of DNA synthesis requirement for the generation of Concanavalin A helper and suppressor cells. J. Exp. Med. 146:747-758.

88. Twomey, J.J., A.H. Laughter, S. Farrow, and C.C. Douglass, 1975. Hodgkin's disease: An immunodepleting and immunosuppressive disorder. J. Clin. Invest. 56:467-472.

89. Waldmann, T.A., M. Dunn, S. Broder, M. Blackman, R.M. Blaese, and W. Strober, 1974. Role of suppressor T cells in pathogenesis of common variable agamma globulinemia. Lancet II:609-614.

90. Waldmann, T.A., and S. Broder, 1977. Suppressor cells in the regulation of the immune response. In: Progress in Clinical Immunology, 3, R.S. Schwartz, ed. Grune and Stratton, Inc., pp. 155-199.

91. Yu, A., H. Watts, N. Jaffe, and R. Parkman, 1977. Concomitant presence of tumor-specific cytotoxic and inhibitor lymphocytes in patients with osteogenic sarcoma. N. Engl. J. Med. 297:121-130.

92. Zembala, M., B. Mytar, T. Popiela, and G.L. Asherson, 1977. Depressed *in vitro* peripheral blood lymphocyte response to mitogens in cancer patients: The role of suppressor cells. Int. J. Cancer 19:605-613.

93. Weiss, B., and W.N. Hait, 1977. Selective cyclic nucleotide phosphodiesterase inhibitors as potential therapeutic agents. Ann. Rev. Pharmacol. Toxicol. 17:441-477.

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