AGE AND GENDER RELATED CHANGES IN NORMAL LEVELS
OF CIRCULATING HUMAN IMMUNE CELLS

Alan A. Waldman, William R. Oleszko, Edith Zang,
Celso Bianco and Johanna Pindyck

The Greater New York Blood Program
New York Blood Center, New York, New York

INTRODUCTION

During the last few years there has been an increase in interest in
the nature and function of circulating human immune cells. This has been
paralleled by increased availability of monoclonal-based reagents which
react with specific cell surface markers, allowing rapid and sensitive
detection of different functional cell types.

With the realization that diagnosis of immune competence might be of
great importance in prediction of susceptibility to diseases, such as
acquired immune deficiency syndrome (AIDS), it becomes necessary to have
data concerning the normal level of circulating immune cells in individuals,
and concerning the extent of acceptable variation in these values. These
data have not been readily available.

Recently, we have begun to examine the circulating immune cell content
of healthy normal blood donors as part of a program designed to establish
the immune status of our donor population. The initial results of the study
demonstrate that the expected values for levels of circulating immune cells
in a healthy adult vary and that the variation is predictable and dependent
on the age, gender, and history of viral infection of the individual.

MATERIALS AND METHODS

Sample Population

All samples were obtained from healthy blood donors who not only passed
the standard medical history, but also attested that they were not a member
of a group with increased risk of exposure to AIDS.

Sample Analysis: Cellular Content

Blood was anticoagulated with potassium EDTA and stored overnight at
room temperature before being analyzed for cellular content. Complete blood
counts were performed using automated cell counters (J. T. Baker, Inc.) and
leukocyte differentials were made on Wright stained smears.

For flow cytometric analysis, samples were first exposed to specific
fluorescein-labeled monoclonal antibodies directed against known cell surface
markers on lymphocytes (Becton Dickinson and Coulter), and then treated
with an NH₄Cl-based lysis buffer to remove erythrocytes (Ortho, Inc.).
The extent of selective staining was determined by examination of the
stained preparation using a flow cytometer (FACS IV, Becton Dickinson).

**Sample Analysis: Serologic Assays**

Serum from a second, matched aliquot of blood was also available for
each donor sample examined for cellular content. The serum was assayed for
the presence and level of IgG antibodies directed against cytomegalovirus
(CMV), using a commercially available fluorescent test (FIAX, IDT Corpora-
tion) and for the total level of the circulating immunoglobulins IgG, IgA
and IgM (ICS, Beckman Instruments).

**RESULTS**

In the first phase of the study, basic screening results were obtained
for almost 1,000 normal, healthy blood donors (627 males, 369 females) with
an age distribution approximating that of the entire donor base. In summary,
the following observations were made concerning the level of circulating
immune cells:

a) For males, the mean percent of lymphocytes bearing the markers for
T helper, inducer cells (T_h,i) increased continually with age of the donor
while the mean percent of lymphocytes bearing markers to T suppressor, cyto-
toxic cells (T_s,c) remained relatively stable;

b) For females, the mean percent of T_h,i also increased continually
with age, and in addition, the mean percent of T_s,c declined substantially;

c) The increase in T_h,i for females was greater than that for males,
as was the decrease in T_s,c; and

d) The net effect of these paired changes was clearly seen in the
mean ratio for T_h,i to T_s,c, with females having on an average a higher
ratio for each group, as well as a much more rapid rate of increase than
did males (p <0.005).

These large and dramatic changes in balances of T_h,i and T_s,c occurred
in the absence of any obvious change in the average level of total circu-
lating white blood cells (WBC) or in the average WBC differential for granu-
locytes or lymphocytes. This was true both for males and for females.

Exposure to, and infection by, cytomegalovirus (CMV) is very common
(1). With increasing age, fewer of the donors exhibited a low (undetectable)
level of IgG antibodies to CMV, and more exhibited a high (greater than 140
fluorescent units) level of antibody to CMV. If one redistributed the data
concerning the percent of T_h,i and T_s,c on the basis of level of antibody
to CMV as well as on the basis of age and gender, one would obtain for both
males and females a slightly lower mean percentage of T_h,i and clearly
elevated mean percentage of T_s,c in individuals with high titers of anti-CMV
compared to individuals with undetectable anti-CMV.

To confirm and extend these initial findings, we examined in greater
detail a second set of donors pre-selected as males or females between 17
and 30 years or 50 and 65 years of age. The results of these studies are
presented in Table 1, 2, and 3.

For both males and females (Table 1), the following parameters were
found to be essentially constant between the younger (<30) and the older
(>50 groups):

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