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AGING AND OBESITY AFFECT THE NUMBER OF B AND NK CELLS IN A PAKISTANI COHORT OF YOUNG AND ELDERLY WOMEN

Rahmat Gul[1,2], Iftikhar Alam[2], Saleem Khan[1], Ibrar Alam[3]
[1]Department of Human Nutrition, the University of Agriculture Peshawar, Pakistan,
[2]Department of Human Nutrition and Dietetics, Bacha Khan University Charsadda, KPK, Pakistan
[3]Department of Biotechnology, Bacha Khan University Charsadda, KPK, Pakistan.

[*]Corresponding author: rgadil@ymail.com

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ABSTRACT

Aging is accompanied by changes in body weight such that the overall immunocompetence is decreased. Insufficient studies are available on the relationships between nutritional status and immune function. The aim of the present study was to investigate the association between nutritional status and B and NK cells in apparently healthy elderly people. Thirty free living elderly, representatively sampled, aged 50 and above participated with a same number of 30 young controls. Anthropometrics (weight, height, body mass index: BMI) were assessed. Venous blood samples were used to asses B and NK cells. The results show slightly more B and NK cells in the elderly as compared to the young; showing an effect of aging. Similarly, there were significantly more B and NK cells in the obese young and the elderly as compared to their normal weight counterparts; showing an effect of weight on the number of these cells. These findings suggest that aging and nutritional status and/or obesity are the two determinants of immune status. Further studies are required to clarify the interplay between nutritional status and/or obesity with particular emphasis of studying subsets of these lymphocytes.

Key Words: aging, obesity, nutritional status, B cells, NK cells, Pakistan.

INTRODUCTION

It is well demonstrated that immune-competence declines with age (Miyaji et al., 2000; Borrego et al., 1999). With normal aging the immune system begins to lose some of its functions and cannot respond as quickly or efficiently to stimuli. Age related changes in the immune system have been observed at all levels ranging from chemical changes within the cells, to differences in the kinds of proteins found on the cells surface, and even to alterations in the entire organs. Some of these changes may seem trivial, but when all of the changes are added up, they radically affect the overall health of the particular individual. Besides other factors, number of cells of immune system is also alters with aging. In the same way, the activity of B and NK cells are reduced in the elderly, although there is very little data available on the effects of aging on these cells types.

Despite a huge number of studies on the relationship between immunity and aging, there are many descriptions of changes in the leukocyte subpopulations with normal aging, and these are not always comparable. A progressive age-related shift in the circulating lymphocyte population from conventional T cells to NK cells is well documented (Facchini et al., 1987), although not a universal finding (Benders et al., 1986). Conflicting data have also been reported with regard to the functional activity of NK cells during aging unchanged (Sansoni et al., 1993; Lighthart *et al.,* 1989; Krishnaraj, 1992), decreased (Fachhini *et al.,* 1987; Miyaji *et al.,* 1997; Ogata *et al.,* 1997; Mariani *et al.,* 1994), or increased (Kutza *et al.,* 1994)]. Possible explanations for these discrepancies may be differences in donor selection criteria and sample size of studies (Facchini *et al.,* 1987; Sansoni et al., 1993). However, abnormalities of NK cell function with age might also be related to micro environmental changes and primarily to endocrine and nutritional factors (Mariani *et al.,* 1998).

Excess weight contributes to several health-related complications (Flegal et al., 2005). It is possible that excess weight may also have a negative effect on immune cell counts. The impact of weight on immune cells, to date, has largely been evaluated with variable results; some suggesting that being overweight or obese is associated with higher immune cell levels (Womack et al., 2007) and others showing that obesity results in decreased immune counts and function (Lynch et al., 2009). It is known that obesity is associated with higher rates of infectious complications, severity of certain viral infections (e.g., 2009 H1N1 influenza A and hepatitis C

virus), and poorer vaccine responses (Eliakim et al., 2006), supporting its potential detrimental effects on immune responses.

Despite the valuable findings of studies examining obesity and immunity or age and immunity, few, if any, studies have investigated the combined impacts of obesity and age on immune cells. To this end, the aim of the present study is to examine the effect of overweight and obesity on immune cell populations in an aged adult model.

METHODS

SAMPLE LOCATION AND SELECTION

The study was conducted in one of Union Council health facilities of Malakand Agency, Khyber Pakhtunkhwa province (KPK) of Pakistan. A convenience sample of 60 women was selected. The mean age of young and old women were, (26.5; 58.8yrs respectively (range: 23.5-29.5yrs; 50.3-66.6yrs respectively). The inclusion criteria were: all subjects were healthy, non-diabetic, with no CVD, and without any recent past history of infection.

ANTHROPOMETRICS AND BLOOD SAMPLE COLLECTION

General anthropometrics were measured. Weight was measured to the nearest 0.01Kg on a bathroom scale. Height was measured to the nearest 0.01 cm using an ordinary non-stretchable tap. Dietary intake was assessed using 24 hr dietary recall. Subjects were asked what they had eaten during the previous day starting from the morning breakfast till the last meal before going to bed. For enumeration of B and NK cells, three ml of blood were withdrawn from each healthy donor under complete aseptic conditions. They were dispensed into a tubes containing EDTA as anticoagulant for performing complete blood count.

B AND NK CELLS ENUMERATION

Venous blood samples were obtained in EDTA-treated tubes early in the morning, after an overnight fast. Blood specimens were set up on the same day of collection. 100 µL of whole blood was taken and placed in a Wasserman tube. The following FITC and PE conjugated antibody combinations were used: CD3, CD16, CD19, CD56. Ten microlitres of antibody was added to each labelled tube, mixed with a Coulter QPREP machine and then left covered for 60 min at room temperature. Cells were lysed. The specimens were then stored in the dark, overnight at 4 °C. On the next day, cell suspensions were transferred into appropriately labelled Eppendorf tubes (with caps on) and spun at 1200 rpm for 5 min. The supernatant was removed and the cells were resuspended in 10 ml PBS/1% BSA/0.01% NaAz and then 2% paraformaldehyde was added. Enumeration of lymphocyte subsets was done using a flowcytometer (Coulter). Total lymphocyte count was used to obtain the absolute counts of CD3 (total T-cells), CD16-CD56 (NK cells), and CD 19 (B-cells).

STATISTICAL ANALYSIS

All anthropometric measurements, data on nutrient intake and immune cells were made in duplicate and the means of paired values were used in the analyses. The data were statistically analyzed using SAS (Version 7.0. SAS, USA). For comparison of means, two-sample t-test was performed to compare the variables of interest of the two groups. Correlation analyses were also performed to calculate the coefficients of correlation and to establish associations between anthropometric measurements, parameters of body composition, nutrient intake and immune cells. Written informed consents were obtained from all the participants before the start of study.

RESULTS

Table 1 shows the general characteristics of the study subjects. The age of the elderly and young ranged from 50.3 – 66.6 (Mean: 58.8 yrs) and 23.5 – 29.5 yrs (Mean: 26.5 yrs), respectively. There was non-significant difference in the mean values of weight, BMI, WC and % BF between the young and the elderly (p, for all trends >0.05). Mean weight of elderly (66.0 Kg; Range: 49.7 – 84.2 Kg) was not different from the mean weight of young (63.5 Kg; Range: 48.8 -88.8 Kg). BMI, WC and % BF of the elderly and young ranged from 16.1 - 31.4 Kg/m2; 56.0 – 104.4 cm; 7.8 – 37.4 % and 17.3 – 29.5 Kg/m2, 56.3 - 104.2 cm, 4.7 – 36.2 %, respectively. Table 1 also shows the mean intake of selected nutrients (energy and protein). There was non-significant difference in the mean values of protein and energy intake between the elderly and young (p, for all trends >0.05). Energy intake ranged from 1535 – 2895 Kcal/day (elderly) and from 1596 – 3185 Kcal/day (young). Protein intake ranged from 22.9 – 53.3 g/day (elderly) and from 29.6 – 54.5 g/day (young).

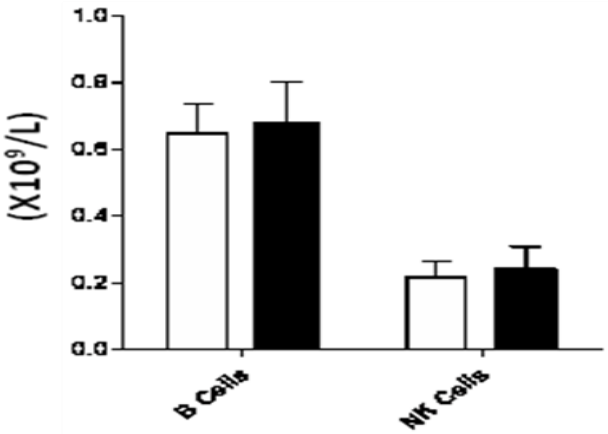

Fig 1: Comparison of B and NK cells between elder and young female. (Blank bar= elder female; black bar= young female)

Table 1: Age, selected anthropometrics and nutrient intake of the subjects

	Old	young	p-value
Age and Anthropometrics			
○ Age (yrs)	58.8	26.8	NS
○ Weight (Kg)	66.0	64.0	NS
○ BMI (Kg/m^2)	23.1	22.7	NS
○ WC (cm)	83.1	84.6	NS
○ % BF	21.7	19.7	NS
Nutrient intake			
○ Protein (g/day)	40.3	39.8	NS
○ Energy(Kcal/day)	2080	2240	NS

NS=non-significant by Student's t-test taking p≤0.05

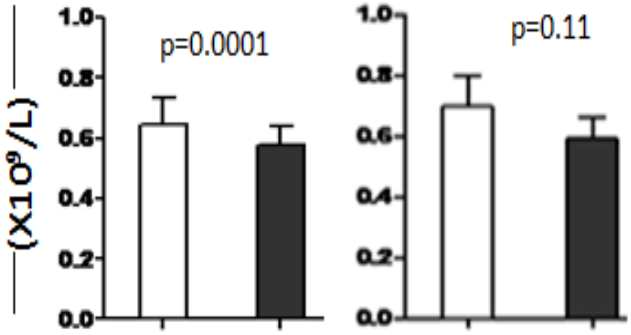

Fig 2: B cells in the two BMI categories of young (A) and elderly (B). Blank and black bars represent obese (OB) and normal weight (NW), respectively.

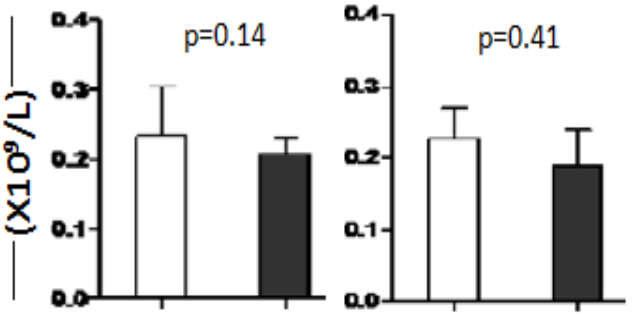

Fig 3: NK cells in the two BMI categories of young (A) and elderly (B). Blank and black bars represent obese (OB) and normal weight (NW), respectively.

NUMBER OF B AND NK CELLS IN YOUNG VERSUS OLD

There were more B and NK cells in the elderly compared to young (0.68 x10^9/L Vs 0.65 x10^9/L and 0.0.24 x10^9/L Vs 0.22 x10^9/L, respectively). However, these differences were not statistically significant (p, for all trends >0.05).

Obese (OB) young had significantly more B cells as compared to normal weight young (0.65x 10^9 /L and 0.71 x 10^9 /L Vs 0.57 x 10^9 /L; p<0.0001). Similarly, OB old had higher numbers of B cells (0.69 x 10^9 /L) as compared to those of normal weight (0.59 x10^9 /L).

Regarding the number of NK cells, OB young had more NK cells compared to NW young (0.24x 10^9 /L Vs 0.21x10^9 /L; p=0.14). Also, OB elderly had more NK cells as compared to the NW elderly (0.23x10^9 /L Vs 0.19x10^9/L, respectively). These differences were, non-significant (p=0.41).

DISCUSSION

In the present study, we notice more B and NK cells in the elderly as compared to the young (Fig. 1). Also, young and elderly obese individuals had higher number of B and NK cells as compared to their normal-weight counterparts (Figs. 2 and 3). Importantly, the differences were significant in young but not in the elderly group. These results show somewhat a relationship between aging and nutritional status with B and NK cells. The same results were reported by Carmen et al (2014), who reported an expansion of CD56−CD16+ NK cells in obese individuals. As obesity is a chronic inflammation and it is likely that decrease of some NK cell subset might be because of the obesity-induced inflammatory status (Carmen *et al.,* 2014).

Aging affects all cell components of the immune system, including B and NK cells. In particular, unlike T and B cells, the absolute number of NK cells increase in aged individuals (Miyaji et al., 2000; Borrego et al., 1999). In a study by Facchini et al. (1987), they found old subjects (71–95 y) had a progressively higher number of NK cells than did young control subjects. However, NK cell cytotoxicity and IFN-γ production, on a 'per cell' basis, are decreased in old age (Borrego et al., 1999). NK cells are able to directly kill cells by releasing perforin and granzymes. These enzymes activate caspases and induce apoptosis of the target cell. As we also noted in our results, the peripheral B cell numbers do not substantially decline with age. Similar to the T cell pool, the peripheral B cell pool fills up with antigen-experienced memory cells at the expense of a concurrent displacement of naïve B cells. The percentage of naïve B cells, which are defined by the absence of CD27, is significantly reduced in aged individuals. In contrast, memory B cells which show a decreased susceptibility to apoptosis (Chong et al., 2005) accumulate in elderly persons, leading to clonal expansions of certain B cell specificities (Chong et al., 2005; Weksler and Szabo, 2000). It has also been speculated that the increase in the number of NK cells with age could be a compensatory mechanism for coping with the reduced cytolytic activity of single NK cells (Facchini et al., 1987; Sansoni et al., 1993).

The analyses presented in the present study are predicated on a well established fact that aging and an assumption that obesity, defined by BMI>24.9Kg/m^2, are determinants of immunocompetence (in the present case B and NK cells). The elderly represent a unique subset of the population with limited nutritional and physiological reserve capacities. Both cross-sectional and longitudinal

studies have demonstrated that there is a linear decline of lean mass and accumulation of body fat with aging (Schwartz et al., 1991; Kullo et al., 2002). The current literature suggests a possible role for cytokines like interleukin-1 (1L- 1) and tumour necrosis factor (TNF) in lean body mass reduction in the elderly (Dinarello and Mier, 1987; Roubenoff et al., 1992; Roubenoff et al., 1993; Sane et al., 1988). On the other hand, current immuno-epidemiological data are insufficient to support this view. Whether there is a linear decline of certain lymphocyte subsets such as B and NK cells, with aging, will require an age-related community based immuno-epidemiological study, preferably longitudinal. These preliminary findings provide an indication that fatness has beneficial effects on certain lymphocyte subsets in elderly women, since total body fat had positive correlations with B and NK cell counts. Such results have also been reported by Widjaja et al (1995).

Nutritional status has been shown to mediate changes in B and NK cells (Mazari et al., 1998), because the elderly tend to have a high prevalence of micronutrient deficiencies (Lesourd and Mazari, 1999; Ravaglia et al., 2000), it is likely they will show these changes more profoundly. Research has shown that obese adults, (BMI≥30 Kg/m^2), have elevated total leukocytes (Niet et al., 1992; Schwaz et al., 1991). The majority of the elevation of leukocytes appears to be related to monocytes (Kullo and Weiss, 1991); however, neutrophils, eosinophils, and lymphocytes may be elevated (Nieman et al., 1999). In addition, several groups have observed that obese adults have elevated levels of many pro-inflammatory Cytokines (Bach-Ngohou et al., 2002; Bullo et al., 2002a; Bullo et al., 2002b; Loffreda et al, 1998; Yudkin et al., 1999). Taken together, these studies suggest that changes in the number of circulating leukocytes in adults reflect some stimulation of stress/inflammatory mediators, such that obesity should be considered a low-level inflammatory condition (Corica et al., 1999; Das, 2002).

NK lymphocytes are innate immune cells that control intracellular infectious agents and cancers. In contrast to T and B lymphocytes, NK cell number is relatively increased in healthy aging and defects in NK cell function are subtle (Lutz et al., 2011; Facchini et al., 1987). As a preliminary exploration of this potential relationship, Lutz, Quinn (2012), studied body mass index (BMI) and NK cells in healthy young and elderly women. In the elderly cohort, BMI correlated inversely with percentage NK cells. These relationships were not seen in young women. As is typical for this age group, elderly subjects had a relatively high percentage NK cells among blood mononuclear cells, which is due both to higher NK cell number and to lower numbers of other blood lymphocytes. This may be a protective mechanism in the elderly when other leukocyte functions decline (Shaw et al., 2010; Larbi et al., 2008; Wikby et al., 2002). These data may suggest that the relative increase in NK cells with aging is blunted by obesity.

The conclusion of the present study is that age and nutritional status have influence on the number of B and NK cells. The effects of obesity on the number of these cells indicate obesity to be a state of inflammation.

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