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Age Related Changes in Fas (CD95) and Fas Ligand Gene Expression and Cytokine Profiles in Healthy Indians

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SUMMARY Ageing in human and animal models show changes in many aspects of protective immunity, particularly lymphopoenia and progressive decline in immune functions leading to increased frequency of infection and neoplasia. However, the exact mechanism of these defects is still unclear. In this study, elderly subjects showed a decline in $CD3^+$ and $CD4^+$ T-cell subsets as well as serum IL-2 levels. Serum IL-6 was significantly raised while expression of its signaling receptor gp130 was significantly impaired in elderly as compared to the younger ones. Additionally, all the elderly individuals showed constitutive expression of Fas and FasL mRNA; however, none of the younger individuals expressed mRNA transcripts constitutively although induced expression was seen in both the groups. Similarly, frequency of Fas and FasL expressing $CD4^+$ and $CD8^+$ T-cell subsets were significantly ($p < 0.001$) higher in elderly subjects as compared to the younger ones. Elderly individuals also showed a significantly ($p < 0.001$) higher frequency of activation induced cell death (AICD). Since interaction of Fas with its cognate ligand (FasL) activates death inducing caspases leading to apoptosis, and gp130 induces anti-apoptotic signal through STAT-3 pathway, these results suggest that the decline in protective immune functions in aged individuals may be related to Fas and FasL mediated apoptosis of peripheral T-cell subsets.

Immune cells offer a powerful model to study effect of aging on gene expression, cell-cell interaction and homeostatic regulation.[1] Immunosenescence is an age-associated deterioration of immune function seen in elderly, which is manifested in increased susceptibility to infection, neoplasia and autoimmune diseases. Aged human and rodents show decline in many aspects of protective immunity, including formation of high affinity antibodies, generation of memory cells after vaccination,[2] delayed-type hypersensitivity response and modulation of Th-like cytokine response such as reduced IFN-γ and IL-2 production as well as IL-2R expression.[1,3] Such dysregulation of lymphocyte functions are thought to contribute to many age-associated defects such as defects in cellular signaling, stem cell and bone marrow defects leading to altered hematopoiesis, involution of thymus and expression of a variety of molecules including hormones and growth

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factors. Ageing is frequently associated lymphopenia leading to progressive decline in T-cell functions due to increased apoptosis of T-cells in ageing humans.[4-6]

In the immune system, apoptosis plays an important role in maintaining T-cell repertoire and regulation of immune response by deleting auto-reactive and activated T-cells after cessation of the activation stimuli.[7] A number of genes and their products regulate the process of apoptosis. Fas/ CD95/APO-1 is a member of TNF (tumor necrosis factor) receptor/NGF (nerve growth factor) receptor family[8] that is widely expressed by many different cell types including B-cells, T-cells, hepatocytes and some tumour cells.[9] FasL, which is predominantly expressed on activated T-cells and NK cells, is a type II membrane protein belonging to the TNF/NGF family, and works as a death factor.[10] Cross-linking of monomeric Fas molecules to FasL activates death inducing caspase cascade leading to apoptotic cell death in sensitive Fas bearing cells.[11] Recent studies[4-6] have suggested increased apoptosis in T-cells of the ageing humans, although, reports on age related changes in expression of Th-like cytokines, IL-6, IL-6R (gp130), Fas and FasL are mutually inconsistent and controversial. Moreover, no reports are available on age related changes in major lymphocyte subsets including Fas and FasL expression in Indian subjects. Therefore, the present study was undertaken to observe major lymphocyte subsets in peripheral blood, cytokine profile and expression of Fas and FasL on the major T-cell subsets in ageing Indians.

MATERIALS AND METHODS

Subjects

This study was done on 65 normal healthy individuals including 42 males and 23 females. These subjects were divided into three age groups; i.e. young (20 subjects; 20-29 years); middle (20 subjects; 40-59 years) and elderly (25 subjects; 60-80 years). No subjects were above 80 years of age. Following inclusion and exclusion criteria were strictly followed in selecting these subjects.

Inclusion criteria

All normal healthy individuals of either sex within the specified age groups were included in the study.

Exclusion criteria

The exclusion criteria included: any sickness within past one month (including viral infection, trauma, accident etc); chronic diseases (including autoimmune disease); infections (including gastro-intestinal tract infection); communicable diseases (within 6 months); major surgery (within 6 months); allergy to drugs or any other substances; pregnancy/ Lactation (within 6 months); vaccination (within 6 months); blood transfusion (within 3 months); active drug and chronic tobacco or alcohol abuse.

None of the subject was using immunosuppressive therapeutic drugs. The appropriate review committee of the All India Institute of Medical Sciences approved the study protocol.

Methods

Heparinized peripheral blood was collected from each individual. Three hundred microliters whole blood was used for immunophenotyping of major lymphocyte subsets by flow cytometry and remaining for isolation of peripheral blood mononuclear cells (PBMC).

Immunophenotyping of cell surface markers by flow cytometry

Two-color flow cytometry was used for immunophenotyping of major lymphocyte subsets. Briefly, 100 µl aliquots of whole blood was taken in three different Eppendorf vials containing 20 µl of fluorochrome labeled respective monoclonal antibodies (Pharmingen, USA), i.e. CD3.FITC/CD56.PE; CD4.FITC/CD8.PE; and anti-mouse lgG2a.FITC/ lgG1.PE was used as isotype control. Cells were incubated in the dark at 25ºC for 30 minutes, washed twice with phosphate buffered saline, pH 7.4 (PBS) and re-suspended in FACS lysing solution (Pharmingen, USA). Cells were further incubated at 37°C for 5 minutes, washed twice with PBS and re-suspended in 1% paraformaldehyde. At this stage the samples were stored at 4°C in the dark until used for flow cytometry.

Isolation of PBMC and culture

PBMC were isolated by Ficoll-hypaque (SIGMA-Aldrich, St. Louis, MO) density gradient

centrifugation. The cells were washed twice with PBS, counted in a hemocytometer and viability was determined by trypan blue dye exclusion test. Viability of the cells was above 95%. Cells were re-suspended at a concentration of 1 x 10^6 cells/ml in RPMI-1640 medium containing 10% fetal calf serum (FCS, SIGMA-Aldrich, St. Louis, MO) and antibiotics. Cell suspension was divided into two aliquots. One aliquot was stimulated with optimum dose of phytohaemagglutinin (PHA, 5 μg/ml) for 48 hours while other aliquot received equal volume of PBS and served as un-stimulated controls. The stimulated cells were further subjected to apoptotic signal by activation with phorbol myristate acetate (PMA, 10 μg/ml) and ionomycin (500 μg/ml) for 6 hours. After termination of the culture both activated and control cells were divided into two aliquots, each. One aliquot from each sample was processed to observe expression of Fas and FasL at mRNA by RT-PCR while other was used to examine expression of Fas and FasL on T-cell subsets at protein level by flow cytometry.

Activation induced apoptosis was determined by flow cytometry using the forward scattering (FSC) and side scattering (SSC) parameters of the *in vitro* activated cells. Lymphocytes were gated on the basis of their FSC and SSC parameters. Gated lymphocytes with lower FSC was considered as apoptotic cells.

ELISA for serum IL-2, IL-4, IL-6, IL-10 and gp130

Th-like cytokine profile such as IL-2, IL-4, IL-6, IL-10 and IL-6 signaling molecule (gp130) in serum was assayed using commercially available ELISA kits (IL-2 & IL-4, Cytimmune Sc. Inc., MD, USA; IL-10, BD-Pharmingen, LA, USA; and IL-6 and gp130, Biosource International Inc., Calif., USA). The assays were performed as per manufacturer's instructions.

RT-PCR for Fas and FasL

Total cellular RNA was extracted from 5 x 10^6 cell pellet by using Tri-reagent (SIGMA-Aldrich) as described earlier,[12] re-suspended in 50 μl of diethylpyrocarbonate (DEPC) treated water and kept at -70°C until further use. Eight microliters aliquot of total cellular RNA was taken and to it 1 μl of DNase I (1 U/ml) was added and the reaction mixture was incubated at room temperature for 15 minutes. The enzyme was inactivated by adding 1 μl of 25 mM ethylene diamine tetra acetic acid (EDTA) and heating at 65°C for 15 minutes. It was followed by brief centrifugation. This DNA free total cellular RNA was used for reverse transcription (RT) reaction.

Reverse transcription of total cellular RNA was carried out using Revert Aid^{TM} H MINUS First Strand cDNA Synthesis kit (MBI Fermentas, USA). Briefly, 2 μg of total cellular RNA was added to 1 μl of Oligo (dT) primer (0.5 μg/μl) and the reaction mixture was reconstituted to 12 μl using deionized nuclease free water. The reaction mixture was mixed properly by gentle centrifugation and incubated at 70°C for 10 minutes and quickly chilled on ice. RT-cocktail was prepared by adding 4 μl 5x reaction buffer, 1 μl RNase inhibitor (20 U/ml) and 2 μl of each 10 mM dNTP. The reaction mixture was centrifuged briefly and tubes were incubated at 37°C for 5 minutes. One microliter of Moloney murine leukemia virus-reverse transcriptase (M-MuLVRT, 200 U/μl) was added to the tubes and it was further incubated at 42°C for 60 minutes. Heating at 70°C for 10 minutes arrested the reaction. The tube was spun down briefly and quickly chilled on ice and PCR amplification was performed for Fas, FasL and β-actin (the positive control) using gene specific primers. The primer pairs used for PCR amplification were as follows: Fas- forward- 5'-CAA GTG ACT GAC ATC AAC TCC- 3', reverse- 5'-CCT TGG TTT TCC TTT CTG TGC-3' (annealing temperature 52°C and product size 548 bp); FasL- forward- 5'- GTT TGC TGG GGC TGG CCT GACT-3', reverse- 5'-GGA AAG AAT CCC AAA GTG CTTC-3' (annealing temperature 58°C and product size 750 bp); β-actin- forward- 5' TGA CGG GCT CAC CCA ACA TGT GCC CAT CTA-3', reverse- 5'- CTA GAA GCA TTG CGG TGG ACG ATG GAG GG-3' (annealing temperature 60°C and product size 658 bp). cDNA amplicons were electrophoresed in 1.2% agarose gel stained with ethidium bromide and visualized under UV transilluminator.

Expression of Fas and FasL at protein level was examined on control and activated T-cell subsets

by two-color flow cytometry using FITC-labeled mAb to respective cell surface markers (CD3, CD4 and CD8) and PE labeled mAb to Fas and FasL (Diaclone, SAS, France) along with appropriate isotype controls. Samples were acquired in Flow Cytometer (FACS Calibur, Becton-Dickinson, USA) and the data was analyzed using CELL QUEST (Becton-Dickinson, USA) software.

Statistical analyses

Statistical analyses of the data was done using EpiInfo version 6.04 d software. One- way ANOVA (Analysis of Variance) and paired Student's t-test was performed for analyzing statistical significance of the data.

RESULTS

Major lymphocyte subsets in peripheral blood

$CD3^+$, $CD4^+$, $CD8^+$ and $CD56^+$ NK cells were analyzed in peripheral blood of the subjects by two-colour flow cytometry. Isotype antibody was used for non-specific fluorescence and for setting the quadrants. In this study none of the samples showed non-specific fluorescence. The results are shown in Table 1. A gradual decrease of $CD3^+$ T-cell subset was observed with advancing age group. Elderly subject showed significantly ($p < 0.041$) reduced $CD3^+$ T-cells as compared to the younger subjects. Similarly, $CD4^+$ T-cell subsets were also gradually reduced with advancing age showing a significant ($p < 0.001$) difference between young (mean ± SD = 40 ± 8) and elderly (mean ± SD = 28 ± 7) groups. $CD8^+$ T-cells were slightly higher in elderly but the difference between young and elderly was not significant. No significant change was found in $CD56^+$ NK-cell subset in relation to age.

Th-like cytokines, IL-6 and its signaling molecule (gp130) in peripheral blood

Serum levels of IL-2, IL-4, IL-10, IL-6 and soluble IL-6 receptor (gp130) was determined by ELISA and results are shown in Table 2. Serum IL-2 showed a gradual decrease with advancing age and significantly low mean value in middle ($p < 0.02$) and advanced age group ($p < 0.001$) as compared to young ones. No significant change was observed in case with IL-4 and IL-10. Serum IL-6 level was significantly higher ($p < 0.001$) in elderly subjects as compared to younger and middle age groups. A gradual decrease was observed in soluble gp130 with advancing age, which was significantly lower in elderly ($p < 0.01$) as compared to the young age group.

Fas and FasL mRNA expression

Fas and FasL mRNA expression was observed by RT-PCR (Fig. 1) at basal level and in activated PBMC of all the subjects. Constitutive expres-

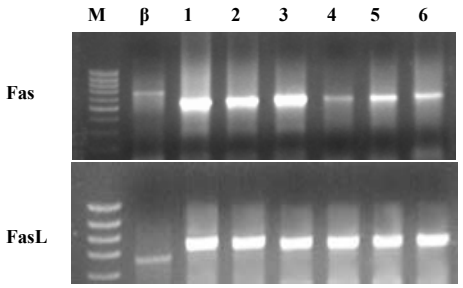

Fig. 1 Representative gel showing induced (lanes 1-3) and constitutive (lanes 4-6) expression of Fas (548 bp) and FasL (750 bp) genes in elderly individuals (lanes 1-6). 1 Kb DNA ladder was used as a molecular weight marker (lane M) and β-actin was used as a positive control (lane β).

Table 1 Major lymphocyte subsets in the different age groups

Age groups	% CD3+ (Mean ± SD)	% CD4+ (Mean ± SD)	% CD8+ (Mean ± SD)	% CD56+ (Mean ± SD)
Young (20)	68.0 ± 10.4	39.7 ± 7.8	31.7 ± 7.3	13.5 ± 6.0
Middle (20)	65.5 ± 10.0	35.4 ± 7.0	28.0 ± 5.8	12.5 ± 7.3
Elderly (25)	60.4 ± 9.5*	28.2 ± 6.5**	34.4 ± 5.5	14.4 ± 5.5

Number in parenthesis indicates number of subjects; *$p < 0.05$; **$p < 0.001$.

sion of Fas was found in all the subjects of middle and elderly age groups but not in the younger subjects. However, induced expression was found in all the age groups. FasL is generally expressed on activated lymphocytes in normal individuals; however, in the present study 50% of the individuals in middle age group and all the aging subjects expressed FasL constitutively while no FasL mRNA was observed in the younger subjects (Table 3). Induced expression of FasL was observed in all the subjects.

Fas and FasL expression in T-cell subsets

In order to determine the quantitative expression of Fas and FasL on T-cell subsets, two color flow cytometric estimation (Fig. 2) was done using CD4/CD8 FITC and Fas/FasL PE monoclonal antibody and appropriate isotype control antibody. Fas expressing $CD4^+$ T-cells in young, middle and elderly age groups were 18 ± 4%, 20 ± 4% and 30 ± 7% (Mean ± SD), respectively, showing a gradual increase with advancing age group (Figs. 2 and 3). Similarly, a gradual increase in Fas expression was observed in $CD8^+$ T-cell subsets from young (16.6 ± 5) through middle (18 ± 10) and elderly (27 ± 14) subjects (Fig. 3). Fas expression was significantly higher both in $CD4^+$ T-cell subsets ($p < 0.001$) and $CD8^+$ T-cell subsets ($p < 0.05$) of elderly individuals as compared to the younger subjects. No significant difference was observed in the frequency of Fas expressing $CD4^+$ and $CD8^+$ T-cell subsets between young and middle age groups.

A significantly higher ($p < 0.001$) proportion of $CD4^+$ T-cells (17 ± 4%) from elderly subjects expressed FasL as compared to the young (10 ± 4%) and middle (6 ± 4%) age group (Fig. 4). Although frequency of FasL expressing $CD8^+$ T-cells was higher in elderly subjects as compared to middle and young aged individuals, the difference was statistically not significant (Fig. 4). When compared between the two T cell subsets, FasL expression was found to be significantly higher in $CD4^+$ T-cells ($p < 0.001$) as compared to $CD8^+$ T-cell subsets, the difference being more prominent in the elderly subjects.

Activation induced apoptosis

Frequency of apoptotic cells in *in vitro* activated PBMC was determined by flow cytometry based on their FSC and SSC parameters in the lymphocyte gate (Fig. 5). Frequency of apoptotic cells was 17.7 ± 3.2% (Mean ± SD) in the younger, 14.5 ± 8.4% in the middle and 48.8 ± 11.0% in the elderly

Table 2 Cytokine profile in different age groups

Age groups	IL-2 (Mean ± SD)	IL-4 (Mean ± SD)	IL-10 (Mean ± SD)	IL-6 (Mean ± SD)	Gp130 (Mean ± SD)
Young (20)	23.7 ± 27.4	4.8 ± 2.6	13.5 ± 4.0	3.0 ± 2.0	598 ± 67
Middle (20)	10.7* ± 10.6	2.7 ± 1.2	12.0 ± 5.0	4.7 ± 2.0	485 ± 63
Elderly (25)	8.7 ± 6.4**	4.0 ± 1.3	13.5 ± 4.0	13.4 ± 4.0**	457 ± 67**

Numbers in parentheses indicate numbers of subjects; *$p < 0.02$; **$p < 0.001$; unit = pg/ml.

Table 3 Fas and FasL mRNA transcripts in different age groups

Age group	Fas positive		FasL positive	
	Basal No. (%)	Stimulated No. (%)	Basal No. (%)	Stimulated No. (%)
Young (20)	0	20 (100)	0	20 (100)
Middle (20)	20 (100)	20 (100)	10 (50)	20 (100)
Elderly (25)	25(100)	25(100)	25(100)	25 (100)

Number in parenthesis indicates number of subjects.

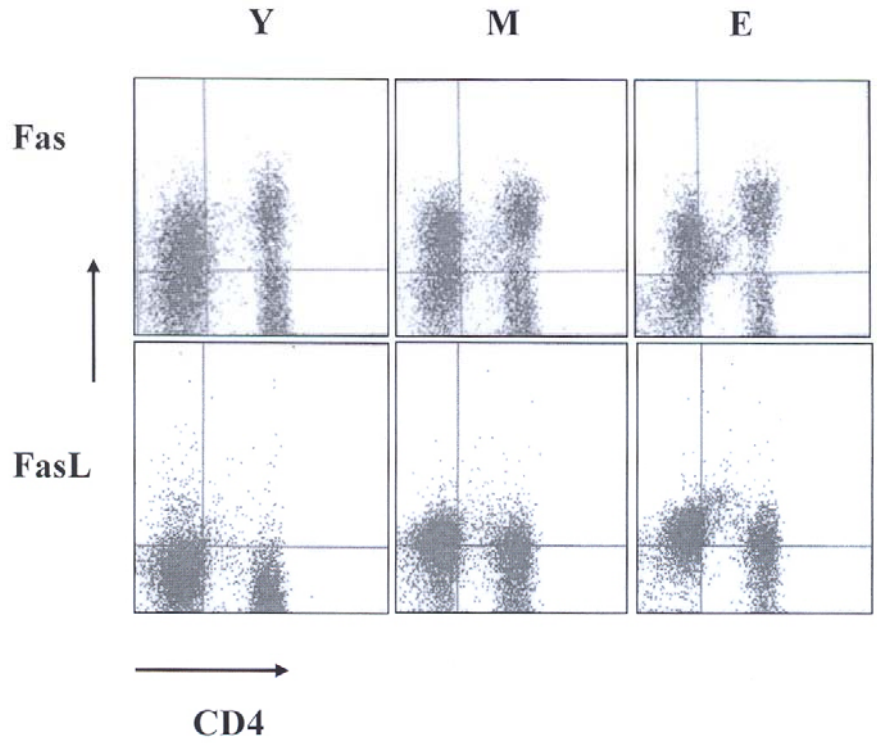

Fig. 2 Representative dot plot showing induced Fas and FasL expression in $CD4^+$ T-cell subsets by two-colour flow cytometry. PBMC were stimulated with PHA and recombinant IL-2 for 48 hours, followed by apoptotic signal provided through PMA and ionomycin for 9 hours.

age groups, respectively (Fig. 6). The results suggest a significantly ($p < 0.001$) higher frequency of activation-induced apoptosis in elderly individuals as compared to the younger ones.

DISCUSSION

Ageing has been found to be associated with defects in some parameters of cellular and humoral immunity, while others such as NK cell activity, phagocytosis and chemotaxis are well preserved in the centenarians.[13-16] Therefore, immunosenescence has been considered as a complex remodeling of the immune system rather than immune deterioration.[16,17] There is a general agreement over decline of T-cell functions with ageing,[1] there are contradictory reports on age related changes in T-cell subsets and NK-cells in peripheral blood of humans and mice.[18,19] In the present study we have shown a progressive decline in the $CD3^+$ and $CD4^+$ T-cell subsets with increasing age, with a significant ($p < 0.001$) difference between young and elderly individuals. Our results are in agreement with earlier reports.[18,20,21] The impaired T-cell population in elderly subjects seems to be related to $CD4^+$ lympho-

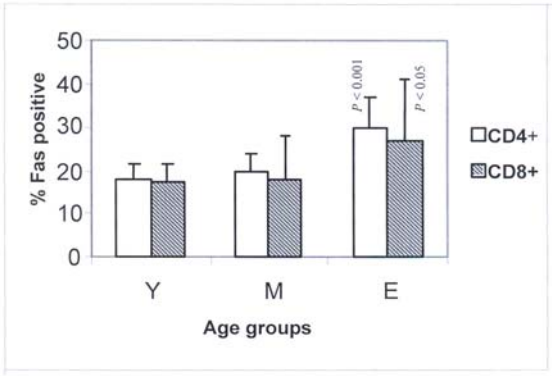

Fig. 3 Fas expression in $CD4^+$ and $CD8^+$ T-cell subsets in young (Y), middle-aged (M) and elderly (E) individuals.

poenia. In this study elderly individuals showed slightly higher frequency of $CD8^+$ T cell subset as compared to the younger and middle age groups. It seems that in the elderly subjects the loss of $CD4^+$ T-cells may be compensated by $CD8^+$ subsets to some extent rather than by the B-lymphocytes. Earlier studies have suggested a decrease in $CD19^+$ cells (B lymphocytes) in the elderly as compared to the younger individuals[15,22] but its function is retained in the centenarians.[15] Functional defects of the T-cells in elderly humans and mice has been found to reflect a shift from naïve to memory T-cell subsets as well as due to alteration in the early events of signal transduction[19,20] and an enhanced TNF induced apoptosis of both naïve ($CD45RA^+$) and memory (CD45RO) T-cells.[23]

Studies on NK-cell function in human and mouse have produced provocative discrepancy. Mouse study with spleen and lymph node derived NK-cells from older animals showed a profound loss

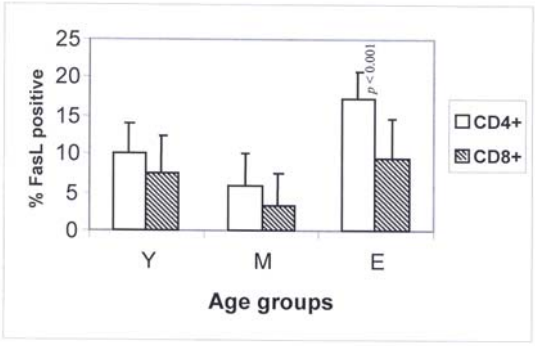

Fig. 4 FasL expression in $CD4^+$ and $CD8^+$ T-cell subsets in young (Y), middle-aged (M) and elderly (E) individuals.

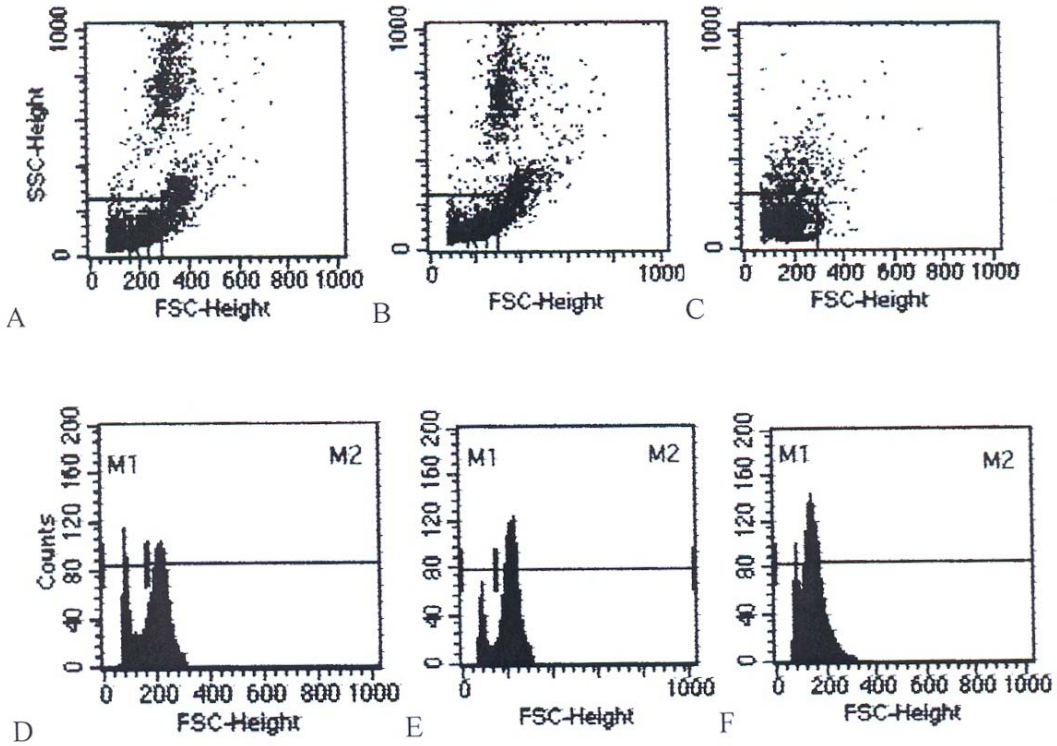

Fig. 5 Representative plots showing apoptotic cells in different age groups. The isolated PBMC were stimulated with PHA and recombinant IL-2 for 48 hours followed by apoptotic signal provided through PMA and ionomycin for 9 hours. Lymphocytes were gated on FSC and SSC parameters (A, B, C) and frequency of apoptotic cells were determined based on FSC parameter of gated lymphocytes (D, E, F).

of NK-cell function, while those derived from peripheral blood of mouse showed no effect of ageing.[1] Tests on human peripheral blood derived NK-cells showed little or no age effect.[13] In the present study no significant change was observed in $CD56^+$ NK-cell numbers in relation to ageing. Our results are consistent with the earlier reports.[13,14] Similarly, majority of studies suggested no difference in NK cell cytotoxicity between younger and elderly individuals.[13] It seems, therefore, that the human peripheral blood NK-cell population is not susceptible to age related changes, however, it may be relevant to see if their cytotoxic function is retained in the elderly individuals. Unless proved otherwise, the contribution of NK-cells in increased sensitivity to neoplasia or viral infections in older people will remain speculative. It has not been widely accepted that the increased rate of cancer in the older population is primarily due to a declining immune functions. One school of thought is that the ageing allows accumulation of mutations in the somatic cells and once it reaches a critical threshold, the affected cells become cancerous.[24]

Unbalance of Th-like cytokine response have been widely implicated in the development of many pathological conditions such as infection, allergy, cancer and autoimmune disorders however; conflicting reports exist regarding cytokine profiles of aged individuals. In ageing human IL-4 in peripheral blood lymphocytes was reduced[25] while increased in mouse spleen with ageing.[26,27] In the present study, a progressive decline in the serum IL-2 level was observed in individuals with the advancing age. Although serum IL-4 and IL-10 levels remained unchanged, serum IL-6 was significantly higher in the elderly subjects. It seems that ageing is associated with a significant impairment of Th1-type of protective cellular immune response and skewing of Th-like cytokine response towards Th2 type. IFN-γ mRNA was reported to be reduced in subjects with the advanced age.[27]

Age related changes in B-cell responsiveness have been attributed to the cellular collaboration necessary for humoral immunity.[20,28] IL-6 is known to be a B-cell differentiation factor that acts through its signal transduction receptor molecule, gp130. It also shows functional redundancy with other cytokines. IL-6 family of cytokines all share gp130 as a

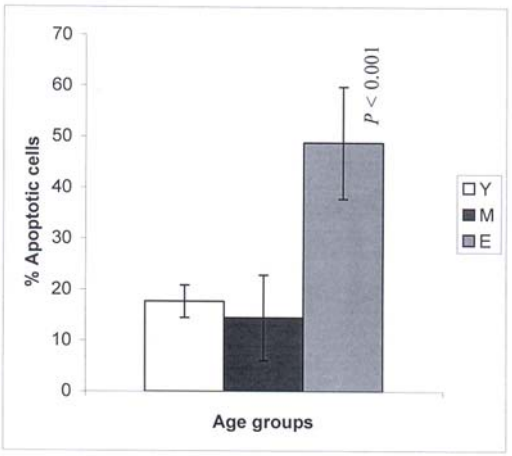

Fig. 6 Frequency of apoptotic cells in the different age groups (Y = young; M = Middle and E = Elderly).

component critical for signal transduction. Downstream of gp130 signaling a variety of events take place including activation of gp130 associated cytoplasmic tyrosine kinase and modification of transcription factors. Signal from gp130 plays an important role in expansion and differentiation of the haematopoietic cells. In the present study, elderly subjects showed significantly higher serum levels of IL-6. Contrarily, soluble gp130 showed a decreasing trend with advancing age. Similarly, women in seventh decade of life were found to express ten fold high amount of IL-6 and higher levels of gp130.[29] In the mouse model, susceptible strain of autoimmune disorders, *MRL/Lpr* mice were reported[30] to have increased levels of IL-6 signaling receptor component gp130. IL-6 and gp130 has been shown to act as growth factors for different tumour cells. IL-6 upon binding to gp130 may induce anti apoptotic signal through activation of STAT 3 leading to expression of *bcl-2* gene.[31] Thus, STAT 3 plays a pivotal role in gp130 mediated signal transduction. However, set of signaling pathway that is activated in a given cell may differ, depending upon the expression patterns of these signaling molecules.[32] On the other hand, gp130 signaling is involved in the differentiation of committed haematopoeitic progenitor cells in the bone marrow, liver and spleen. Deficiency of gp130 has been related to greatly reduced colony forming units in these organs.[31] It seems that down regulation of gp130 may contribute to the inhibition of anti apoptotic mechanism induced by IL-6 as well as the

defective replenishment of apoptotic cells in the peripheral lymphocytes in aged individuals.

Apoptosis plays an important role in immune homeostasis. Activation-induced apoptosis of T-cells has been reported variously in relation to ageing. Some studies suggested an enhanced[33] while other reported a decreased[34] apoptosis in T-cells with ageing. In the present study PBLs from elderly individuals showed significantly higher ($p < 0.001$) activation induced apoptosis as compared to the younger and middle age groups. Recent report suggested significantly enhanced apoptosis of both $CD4^+$ and $CD8^+$ T-cell subsets in elderly subjects in response to TNF signaling.[18] Since Fas and FasL interaction is one of the major mechanism of apoptosis in activated T-cells, we have examined Fas and FasL expression at mRNA level by RT-PCR and at protein level by flow cytometry in T-cell subsets. At the basal level, Fas and FasL mRNA transcripts were observed in all the elderly subjects but not in the younger ones. In case with activated T-cells these molecules were expressed both in the younger as well as in the elderly subjects. Pattern of expression of Fas and FasL mRNA transcripts has been confirmed at the protein level by flow cytometry. A progressively enhanced expression of Fas and FasL was observed in both $CD4^+$ and $CD8^+$ T-cell subsets with the increasing age with a significant difference between younger and elderly subjects. In addition, $CD4^+$ T-cell subsets in the elderly subjects expressed FasL more frequently as compared to the $CD8^+$ T-cell subsets suggesting its differential expression in different T-cell subsets. In a recent study on centenarians a higher expression of Fas was observed in relation to advancing age but FasL mRNA was significantly down regulated suggesting a lower sensitivity to Fas mediated apoptosis.[16] It seems that there may be a selection process in which subjects who live to the age of 90 are those in whom the least decrease in immune response is demonstrated.[13] In elderly human, increased expression of Fas has been reported in memory $CD4^+$ or $CD8^+$ T-cells[35] leading to increased apoptosis in $CD4^+$ and $CD8^+$ T-cell subsets.[6] Fas expression on thymic epithelial cells has also been implicated in the age-related thymic involution.[36]

The overall results suggest a decline in $CD3^+$, $CD4^+$ T-cell subsets, down regulation of IL-2 and gp130, as well as higher expression of IL-6, Fas and FasL in the elderly subjects. Enhanced expression of Fas and FasL on T-cell subsets seems to be related to apoptosis induced lymphopenia in the aged Indians through sixth to eighth decade of their life. It may be reasonable to presume that a higher secretion of IL-6 but reduced levels of gp130 may contribute to the defective immunoregulatory mechanisms leading to down regulation of anti-apoptotic signaling as well as defective replenishment of apoptotic lymphocytes in the elderly individuals.

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