Balanced Regulation of mRNA Production for Fas and Fas Ligand in Lymphocytes From Centenarians

How the Immune System Starts Its Second Century

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***Background*—**The functionality of the immune system during aging is crucial for protection against the most common age-related diseases. Apoptosis plays a central role in the senescence of the immune system, as evidenced by the increased plasma membrane expression of a key molecule like Fas protein. We analyzed the mRNA levels of different forms of Fas (total [tFas] and membrane [mFas]) and of its ligand (FasL) in peripheral blood lymphocytes from centenarians, the best example of successful aging, who were compared with young and middle-aged donors.

***Methods and Results*—**Using real-time polymerase chain reaction, we quantified mRNA for different forms of Fas and for FasL. In resting lymphocytes, mRNA for tFas, but not for mFas, significantly increases with age, whereas FasL mRNA significantly decreases. In vitro production of Fas/FasL mRNA after different stimuli was similar in cells from the 3 groups. Even if the percentage of Fas^+ cells was higher than in the other groups, peripheral blood lymphocytes from centenarians had normal Fas-induced apoptosis, as revealed by flow cytometry. By ELISA, we observed that cells from centenarians showed normal in vitro production of the soluble form of Fas (sFas) and that plasma levels of such molecule were significantly higher in centenarians than in the other groups.

***Conclusions*—**Lymphocytes from centenarians are able to balance the production of proapoptotic (mFas and FasL) and antiapoptotic (sFas) molecules, whose proportions are likely crucial for the well-preserved immune functionality at the extreme limits of human life. **(*Circulation*. 2004;110:3108-3114.)**

Key Words: aging ■ apoptosis ■ immune system ■ lymphocytes

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Growing attention in the scientific community has been focused on human longevity, and centenarians are the best models of physiologically successful aging in humans. Along with other researchers, we have pioneered work in this field and analyzed clinical, immunological, endocrinological, biochemical, and genetic aspects of physically and cognitively healthy centenarians. Studies on their immune system revealed parameters that follow the degenerative trend often present in aged people (eg, reduction of B and T lymphocytes, reduction of proliferative capability), whereas other parameters are well preserved (natural killer cell activity, chemotaxis, phagocytosis) or even increased (production of proinflammatory cytokines).[1–4] We thus proposed that immunosenescence should be considered a complex remodeling rather than a simple sum of deteriorations.[5]

Apoptosis is a normal, physiological process that through active cell demise leads to removal of mutated or damaged cells. Insufficient apoptosis can contribute to the pathogenesis of cancer, autoimmune disorders, and sustained viral infections, whereas excessive apoptosis results in inadequate cell loss and consequent degenerative disorders such as, for example, Alzheimer disease.[6] Apoptosis is also crucial during physiological aging.[7] The aged organism can accumulate senescent (nonproliferating) cells as a result of replicative senescence, which occurs throughout life in multicellular organisms.[8] One could expect that such cells are exhausted not only by their division potential but also by their capacity to trigger and/or undergo apoptosis. Indeed, it has been shown recently that in vitro senescing human fibroblasts have an increased propensity to resist apoptosis induced by simple withdrawal of serum.[9] Scanty and controversial data exist on immune cells,[10] and recently we showed that peripheral blood lymphocytes (PBL) from healthy centenarians display a consistent capacity to resist apoptosis induced by 2-deoxyribose.[11]

In the immune system, one of the most important apoptotic pathways is triggered by Fas (CD95/Apo-1).[12] Fas is present on the cell surface as a monomeric protein and can be bound

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by its natural ligand, termed Fas ligand (FasL; CD178).[13] Cross-linking of Fas by FasL leads to trimerization of Fas death domain in the inner membrane surface, a phenomenon that recruits different caspases and initiates a complex signaling pathway ultimately leading to apoptosis.[14] Depending on the splicing of a primary RNA transcript,[15] Fas can be produced in 2 main forms, ie, membrane (mFas) and soluble (sFas), that have different activities. After binding FasL, the membrane form triggers apoptosis, whereas the soluble form can block FasL before its arrival to mFas, thereby inhibiting its capacity to induce cell death.[16] Thus, it has been hypothesized that the ratio between the 2 forms could be crucial for the propensity of the cell to undergo apoptosis or to survive.[17]

Growing attention has focused on the role of Fas in human aging and longevity. The number of lymphocytes expressing Fas on the plasma membrane increases with age.[18,19] Along with reduced expression of the antiapoptotic molecule Bcl-2, this has been correlated with the existence of an age-related increased susceptibility to apoptosis by T cells.[20] Molecular genetic studies have investigated the presence of different polymorphisms in Fas and FasL genes in centenarians, who did not differ from young subjects.[21] Little is known concerning the molecular mechanism(s) that are able to modify the expression of Fas or FasL in lymphocytes of elderly subjects or to alter their transcriptional regulation. It is not known whether or how senescence can influence the activity of these genes. By using a recently developed assay, based on real-time polymerase chain reaction (PCR), we investigated the regulation of such genes during human longevity and analyzed the expression of the different forms of Fas and of FasL in a group of centenarians.

Methods

Subjects and Stimulation of PBL

Expression of Fas and FasL and functionality of the Fas pathway were examined in a total of 13 young subjects (aged 19 to 40 years; mean±SD age, 23.5±6.1 years), 13 subjects aged between 57 and 62 years (mean±SD age, 60.2±1.2 years), and 14 subjects aged >100 years (maximum age, 103 years). Men and women were distributed equally within the groups. All subjects were in optimal health conditions and fulfilled the criteria defining a healthy donor suitable for immunological analyses during longevity.[22] All individuals were living in the Emilia-Romagna region of Italy and gave informed consent.

Blood was collected by venipuncture and processed within 2 hours; platelet-free plasma was obtained following standard procedures, aliquoted, and immediately frozen at −80°C until use. Peripheral blood mononuclear cells were obtained by gradient density centrifugation, and monocytes were removed by plastic adhesion according to standard methods. The resulting PBL were then used for quantification of mRNA levels of Fas and FasL and for functional studies. For this purpose, cells were stimulated with anti-CD3 monoclonal antibody (mAb) (20 ng/mL; American Type Culture Collection) or with 3 ng/mL phorbol 12-myristate 13-acetate (TPA) plus 2 μmol/L ionomycin (both from Sigma Inc) for 4, 24, and 48 hours at 37°C in RPMI 1640 medium containing 10% fetal calf serum, 2 mmol/L L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin (complete medium), then collected, extensively washed, and processed. Cell pellets were used for the extraction of RNA and quantification of Fas and FasL messengers; supernatants collected after 24 and 48 hours were used for quantification of sFas.

For the induction of apoptosis, 1×10^6 PBL were treated with anti-CD95 mAb (clone CH11, IgM, 20 ng/mL, from Upstate Biotechnology). Cells were incubated for 48 hours in 1 mL of RPMI 1640 medium added to FCS, then analyzed by flow cytometry.

Real-Time PCR for Quantitative Analysis of mRNA Expression

Total RNA was extracted from PBL with the use of RNeasy Minikit by Qiagen. One microgram of RNA was reverse transcribed with the use of Random hexamers, with RevertAid First Strand cDNA Synthesis Kit by Fermentas. The total volume of reverse transcription reaction was 25 μL; 1/25 of cDNA was used for each amplification. Primers for the whole pool of Fas messengers (total Fas [tFas] composed of both the soluble and membrane forms) were as follows: tFas forward: 5′ TGAAGGACATGGCTTAGAAGTTG-3′; tFas reverse: 5′-GGTGCAAGGGTCACAGTGTT-3′; tFas probe: 5′-(FAM)-AAACTGCACCCGGACCCAGAATACC-(BHQ1)-3. The primers for the mRNA exclusively encoding for mFas were as follows: mFas forward: 5′-TCATCAAGGAA-TGGACACTCA-3′; mFas reverse: 5′-GACAAAGCCACCCCA-AGTTA-3′; mFas probe: 5′-(FAM)-CCAGCAACACCAAGTGC-AAAGAGGAAGG-(BHQ1)-3′. The primers for FasL were as follows: FasL forward: 5′-GCAGCCCTTCAATTACCCAT-3′; FasL reverse: 5′-CAGAGGTTGGACAGGGAAGAA-3′; FasL probe: 5′-(FAM)-TCCCCAGATCTACTGGGTGGACAGC-(TAMRA)-3′. The primers for L13/high basic protein (HBP) were as follows: HBP forward: 5′-ACCG-GTAGTGGATCTTGGCTTT-3′; HBP reverse: 5′-GCTGGA-AGTACCAGGCAGTGA-3′; HBP probe: 5′-(Texas Red)- TCTTTCCTCTTCTCCTCCAGGGTGGCT-(BHQ2)-3′. These sets of primers amplify 118 bp of tFas, 101 bp of FasL, 87 bp of mFas (designed to include a portion of the fifth exon of Fas gene, which encodes for the transmembrane domain), and 104 bp of HBP. The primers were synthesized by Operon Qiagen. A portion of each cDNA was used for quantitative PCR in 50 μL of PCR master mix consisting of PCR buffer 1X (Promega), $MgCl_2$ 3 mmol/L, 200 nmol/L of each primer for HBP amplification, dNTPs 200 nmol/L, 2U *Taq* polymerase, HBP probe 300 nmol/L, and tFas, mFas, or FasL probe 300 nmol/L. Each sample was monitored in triplicate for each mRNA. Expression of the messengers was calculated as relative expression referred to 1000 copies of HBP mRNA, on the basis of differences in the threshold cycle of mRNA for tFas, mFas, or FasL and threshold cycle of HBP mRNA, as described.[23]

ELISA for Quantification of sFas

For quantification of sFas in plasma and cell supernatant, an ELISA (sFas Quantikine, R&D System) was used following instructions recommended by the manufacturer.

Flow Cytometry Analysis of CD95 Expression and Functionality

To identify the percentage of Fas^+ PBL, we used flow cytometry according to standard methods. To better distinguish and quantify at the protein level the expression of Fas,[19] we analyzed separately helper or cytotoxic T cells, selecting those that did not express the CD45RA molecule (which is mainly, but not only, present on virgin T lymphocytes). We used anti-Fas, anti-CD45RA, anti-CD4, or anti-CD8 mAbs (from Serotec, directly conjugated with phycoerythrin (PE), fluorescein isothiocyanate, and allophycocyanine, respectively), and a CyFlow ML flow cytometer (Partec, Germany), as described.[24] Data were acquired with the use of a logarithmic amplification of the photomultiplier devoted to collect the fluorescence from PE. Then data were transformed into a linear scale, as described elsewhere.[11] We took the linearized median value of the positive cells in each sample and compared it with the fluorescence value of Fas-negative cells present in the same sample (that typically were $CD45RA^+$). The difference between these 2 values, ie, the relative fluorescence intensity of stained cells, indicates the number of linear channels representing the net fluorescence due to the binding of the mAb to its antigen and thus the amount of antigen. Autofluorescence from the cell populations we analyzed was always negligible (not shown).

Apoptosis was evaluated by the appearance of the hypodiploid peak of propidium iodide fluorescence and changes in physical parameters, as described.[25] Briefly, after a 48-hour incubation with anti-CD95 mAb, cells were resuspended in hypotonic solution containing 0.1% sodium citrate, 0.1% Triton X-100, and 50 μg/mL propidium iodide and kept for 20 minutes at 4°C. Thereafter, cells were analyzed, and those with low propidium iodide fluorescence and an increased side scatter were considered apoptotic.

Statistical Analysis

Statistical analysis was performed with the use of GraphPad Prism software 3.03 under Windows XP. Differences in tFas, mFas, and FasL mRNA levels and differences in sFas protein levels at different ages between 2 groups of subjects were evaluated with the use of 1-way ANOVA test with Tukey posttest. ANOVA with posttest for linear trend was also performed to analyze the global age-related trend. Changes in the mRNA levels and sFas in culture medium after lymphocyte stimulation were evaluated with ANOVA test for repeated measures, with Dunnett posttest. Differences were considered significant when $P<0.05$.

Results

Analysis of Fas and FasL mRNA Basal Levels in Young and Middle-Aged Subjects and Centenarians

We first analyzed basal expression of tFas and mFas forms of Fas and of FasL mRNA in lymphocytes from young, middle-aged, and centenarian donors. We used an approach based on real-time PCR that allowed us to normalize the mRNA levels for such molecules. Indeed, all data have been related to 1000 copies of mRNA for a reporter gene, ie, HBP.[23] We observed an age-dependent, linear increase in tFas mRNA levels, with mean values of 5.08±1.03 copies per 1000 copies HBP mRNA for young, 7.19±1.51 copies for middle-aged, and 15.48±2.96 copies for centenarian subjects ($P<0.02$ versus young donors and $P<0.05$ versus middle-aged donors). This tendency was not reflected in the expression of mRNA encoding for mFas. In fact, mFas levels were always lower than those for tFas and remained similar at all ages (P always NS), with mean values of 1.78±0.50 copies for young, 2.72±0.56 copies for middle-aged, and 2.13±0.49 copies for centenarian subjects. As a consequence, when the increase in tFas is considered, there is an age-related decrease in the relative percentage of mRNA for mFas. Centenarians are thus characterized by the prevalence of mRNA for sFas, which can be derived by the difference between tFas and mFas.

Using the same approach, we quantified the expression of FasL mRNA, which showed an opposite, linear trend in comparison with Fas and significantly decreased with age, with mean levels of 24.72±6.94 per 1000 copies HBP mRNA for young subjects, 10.67±3.80 copies for middle-aged subjects, and 3.39±0.70 copies for centenarians ($P<0.01$ versus young donors and $P<0.05$ versus middle-aged donors).

tFas, mFas, and FasL mRNAs Are Inducible in a Similar Manner in All Groups of Donors

To evaluate the functionality of signal transduction pathways that regulate Fas and FasL production after lymphocyte activation, we analyzed Fas and FasL mRNA levels after in vitro stimulation of PBL. Cells were stimulated for 4 hours with anti-CD3 mAb, which mimics the activation mediated by the T-cell receptor, and with TPA/ionomycin, which upregulates FasL via activation of protein kinase C. As shown in Figure 1, mRNA levels for tFas were significantly increased (2- to 3-fold) by anti-CD3 mAb, whereas TPA/ionomycin did not provoke the same effect. The effects of T-cell activation via anti-CD3 mAb were similar in all of the groups studied. A similar result was obtained by a parallel analysis of mFas mRNA levels, suggesting that a generalized, increased transcription of Fas gene occurs (Figure 2).

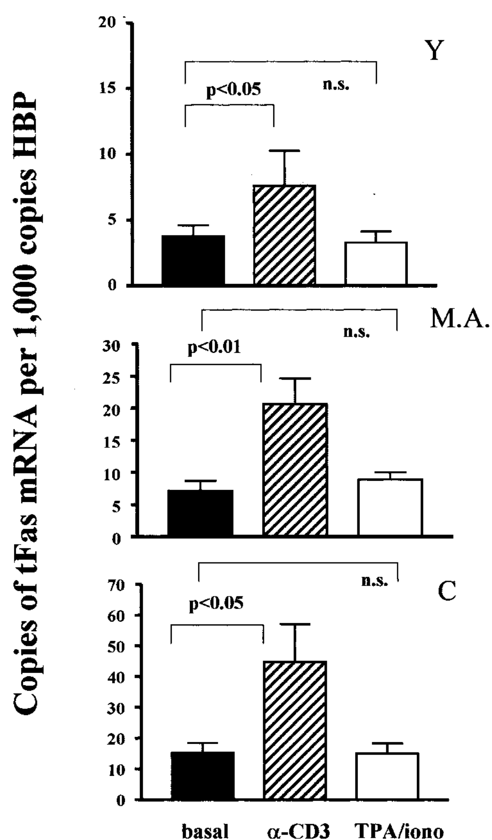

Figure 1. mRNA expressions of tFas in lymphocytes from young (Y) and middle-aged (M.A.) subjects and centenarians (C) without any stimulation (basal) and after stimulation with anti-CD3 mAb and TPA plus ionomycin (iono). mRNA levels are expressed as number of copies per 1000 copies of HBP mRNA. Statistical analysis was performed by ANOVA for repeated measures with Dunnett posttest.

In regard to FasL, mRNA levels were strongly increased by TPA/ionomycin in lymphocytes from young subjects (≈6.3-fold); anti-CD3 mAb led to a less marked (≈3.6-fold) but significant increase as well (Figure 3). A similar trend was observed in middle-aged subjects and centenarians even if, in the latter group, the relative upregulation of FasL obtained by using TPA/ionomycin was much higher (≈12.8-fold) than in the others. In centenarians, adding anti-CD3 resulted in a consistent increase as well (≈6.7-fold).

Quantification and Functionality of Fas by Flow Cytometry

The percentage of Fas^+ T cells is shown in Figure 4 (top). An age-related increase in Fas^+ cells was present among both $CD4^+$ and $CD8^+$ T lymphocytes. However, when the amount

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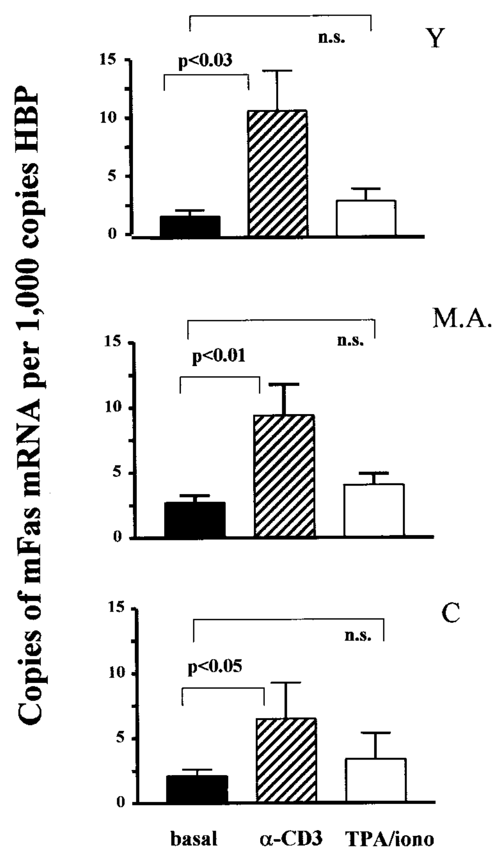

Figure 2. mRNA expressions of mFas in lymphocytes from young (Y) and middle-aged (M.A.) subjects and centenarians (C) without any stimulation (basal) and after stimulation with anti-CD3 mAb and TPA plus ionomycin (iono). mRNA levels are expressed as number of copies per 1000 copies of HBP mRNA. Statistical analysis was performed by ANOVA for repeated measures with Dunnett posttest.

of Fas protein present on CD45RA-negative T lymphocytes was considered, no increase was observed at the single-cell level (middle). Indeed, the analysis of the relative fluorescence intensity of cells stained with PE-labeled anti-Fas mAb, which is directly proportional to the number of antigens present on the plasma membrane, revealed that lymphocytes from the 3 groups were able to bind the same amount of mAb and thus had the same amount of antigen, even if the percentage of Fas$^+$ cells was markedly different.

In regard to the induction of apoptosis in PBL treated with anti-Fas mAb, no differences were found between samples from centenarians and middle-aged and young donors (bottom), indicating that the Fas transduction pathways were functional even at the extreme limit of life.

Analysis of sFas Plasma Levels

Because we observed that tFas mRNA levels increased with age, whereas mRNA for mFas did not, we addressed whether this change was reflected in plasma levels of the soluble form of the protein. sFas levels increased significantly with age, with a level of 4344±746 pg/mL in young subjects, 5888±1476 pg/mL in middle-aged subjects, and 8900±1477 pg/mL in centenarians ($P<0.001$ versus young and $P<0.01$ versus middle-aged donors).

Analysis of sFas Levels in Supernatant of Lymphocytes

Finally, we analyzed the production of sFas by measuring its levels in supernatants of lymphocyte cultures from subjects of different ages. As shown in Figure 5, sFas levels were significantly increased by anti-CD3 after both 24 and 48 hours in all 3 groups ($P<0.05$ after 24 hours and $P<0.01$ after 48 hours in all cases). Ionomycin plus TPA had no or little effect at 24 hours, with a slight increase after 48 hours that was not significant. No significant differences were observed in anti–CD3-induced sFas production between young, middle-aged, and centenarian subjects.

Discussion

Increase in life expectancy at the individual level, along with the rise in mean age of the population, is currently a phenomenon with enormous and dramatic implications of social, economic, epidemiological, and political importance. Age-associated pathologies such as cardiovascular diseases, autoimmunity, cancer, and disabilities have increased accordingly, and we are coping with an unprecedented prevalence of the aforementioned diseases.

It may be relatively easy to identify the environmental conditions that have caused the increase in life expectancy, but it is not simple to understand which biological factors

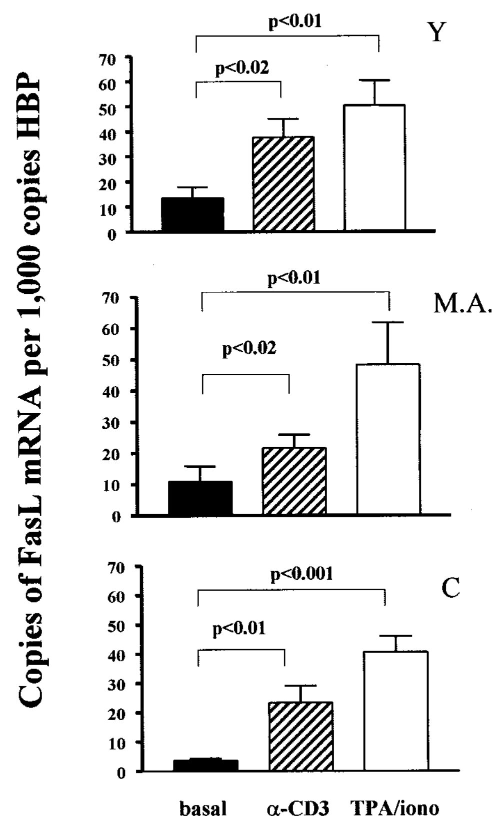

Figure 3. mRNA expressions of FasL in lymphocytes from young (Y) and middle-aged (M.A.) subjects and centenarians (C) without any stimulation (basal) and after stimulation with anti-CD3 mAb and TPA plus ionomycin (iono). mRNA levels are expressed as number of copies per 1000 copies of HBP mRNA. Statistical analysis was performed by ANOVA for repeated measures with Dunnett posttest.

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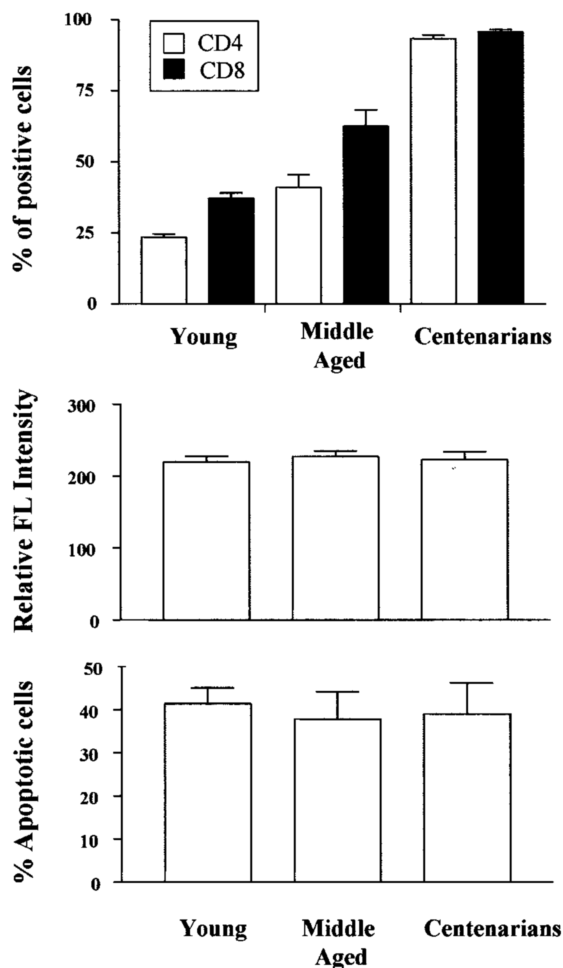

Figure 4. Top, Percentage of Fas$^+$ cells among CD4$^+$ or CD8$^+$ T cells from young donors, middle-aged donors, and centenarians; a statistically significant difference ($P<0.01$) was present between centenarians and young subjects or middle-aged donors in either CD4$^+$ or CD8$^+$ T lymphocytes. Middle, Expression of Fas on the plasma membrane of cells from young subjects, middle-aged subjects, or centenarians; no statistical differences were present among the groups. FL indicates fluorescence. Bottom, PBL tendency to undergo apoptosis (measured as percentage of apoptotic cells after a 48-hour incubation with anti-Fas mAb) after activation of the Fas pathway; no significant differences were present among the groups.

(genetic background and quality of immune responses, among others) are involved in this phenomenon. In the present report we analyzed at mRNA and protein levels the expression of 2 genes that are crucial for homeostasis of the immune system, ie, Fas and FasL, in lymphocytes from centenarians. We found that the expression of tFas, but not mFas, mRNA increases with age. Several studies have described an age-dependent augmentation of lymphocytes expressing Fas on their plasma membrane, reflecting an increase in the number of PBL that switch from naïve to memory phenotype.[18,20] In freshly collected, unstimulated cells, we measured the actual production of such mRNA and found that lymphocytes from centenarians do not produce more mFas mRNA than cells from the other groups. This suggests that basal production of mRNA for mFas remains constant over the years and that the described increase in Fas$^+$ lymphocytes is likely due to their accumulation, not to their new production. This observation supports the theory that considers immunosenescence as the result of the continuous challenge represented by the exposure to several unavoidable antigens (such as viruses, bacteria, food, and self-molecules). The activation of the system and the consequent production of effector/memory cells indicate that the reaction to antigen is successful. This leads to a progressive accumulation of (expanded) clones of memory, Fas$^+$ cells that can fill the entire "immunological space," ie, the physical volume that the body saves for immune cells.[26] Filling of the immunological space, along with the well-known reduced thymic activity, leaves no room for the eventual production of virgin, Fas-negative cells, thereby reducing the possibility to react to other, newly encountered antigens.

The main forms of Fas are the membrane and the soluble forms. Other forms also exist that, however, do not represent >2% of the total[27] and are likely of negligible importance. mFas and sFas are generated by alternative splicing of the primary transcript, depending on the inclusion in the mature messenger of the fifth exon, which encodes for the transmembrane domain.[15] Our data indicate that tFas mRNA levels increase with age but also that there is no parallel increase in mFas mRNA levels. This indicates that the ratio between mRNA for mFas and mRNA for sFas (which cannot be measured directly because it does not contain specific components, but whose levels can be acceptably calculated by subtracting the membrane from the total form) changes with age. Thus, although young subjects show a ratio of approximately 1:1 between the 2 forms, there is an age-dependent

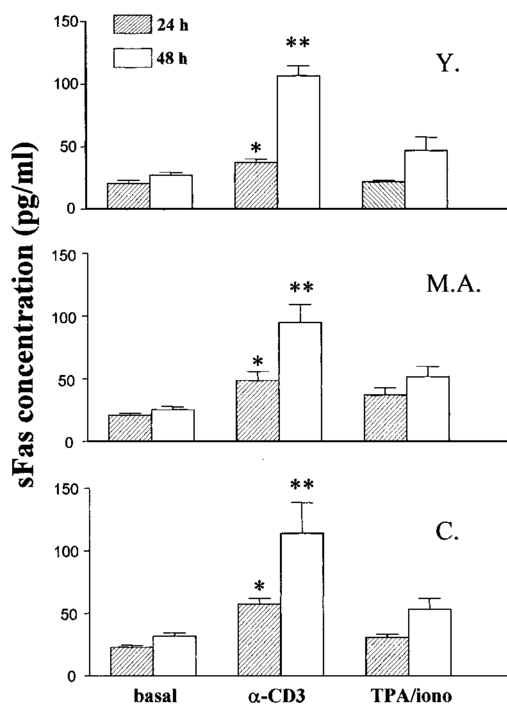

Figure 5. Concentration of sFas in culture media of lymphocytes from subjects of different ages (young [Y.], middle-aged [M.A.], and centenarian [C] subjects) after stimulation with anti-CD3 mAb or TPA/ionomycin (iono) for 24 and 48 hours. Comparisons between groups were performed by ANOVA for repeated measures with Dunnett posttest. *$P<0.05$, **$P<0.01$ vs basal levels at the same time.

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increase in the relative abundance of mRNA for sFas, which becomes prevalent mostly in centenarians. This increase can be interpreted as a protective mechanism against apoptosis, likely occurring to compensate for or prevent an excessive death (or tendency toward death) of lymphocytes from these individuals.

This hypothesis is confirmed by the augmentation of sFas plasma concentration observed with age. Increased plasma sFas levels have been described in patients with chronic obstructive pulmonary disease or in patients with congestive heart failure.[28,29] This observation in centenarians who never had such pathologies is thus similar to the paradoxical rise in proinflammatory cytokine production that we have previously described.[1,5] The increase in sFas plasma levels from 20 to 100 years is not as dramatic as in the case of lymphocyte mRNA production. Other organs, such as liver and kidney, can produce and secrete sFas, and it cannot be excluded that they could contribute more significantly than lymphocytes in determining its plasma level. Studies in animal models indicate that liver is characterized by an age-dependent increase of Fas expression.[30,31] To our knowledge, there are no reports regarding changes of Fas expression or production in liver or kidney (or other organs) with age in humans, and further studies could clarify whether this increase in Fas expression is a general, widespread phenomenon or if it is limited exclusively to lymphoid cells.

We observed a decrease of FasL mRNA levels with age, particularly evident in centenarians. This is in full agreement with data showing an age-dependent decrease in FasL plasma levels.[32] Reduction in the transcription of FasL can be considered another strategy to avoid an excessive depletion of lymphocytes via Fas/FasL apoptosis, the main factor responsible for so-called activation-induced cell death. The increased in vitro susceptibility of lymphocytes from aged donors to Fas-mediated apoptosis, reported in other studies,[33] may be explained at least in part by taking into account that the (excessive?) amount of apoptotic stimuli that are applied in vitro (as well as the dynamics of the phenomenon) likely does not mimic properly the physiological stimuli or the environment where cells live.

Analysis of Fas and FasL expression after stimulation with anti-CD3 mAb and TPA/ionomycin indicates that both genes can be strongly upregulated in lymphocytes from all donors and, at least in the case of Fas, that the messengers are efficiently translated (as indicated by sFas secretion in the culture medium). This suggests that signal transduction mechanisms induced by lymphocyte activation are well preserved during longevity. This is corroborated by observations regarding the expression of Fas at the protein level and its preserved capacity to induce apoptosis in cells from centenarians. However, even if the percentage of Fas$^+$ cells was higher in centenarians, the capacity of PBL to undergo apoptosis was similar to that in young donors or middle-aged subjects, suggesting a lower sensitivity to such apoptotic stimulus. Contrasting data exist in the literature concerning apoptosis and aging. Our results indicate that lymphocytes from centenarians do not have an increased susceptibility to undergo Fas-induced apoptosis. When it is considered that centenarians produce a high amount of sFas, it can be hypothesized that this soluble factor plays an effective role in protecting cells from apoptosis.

The pattern of expression of Fas observed at the mRNA level has been confirmed at the protein level, at least in the case of sFas: there is a significant upregulation of the gene after 24 hours of stimulation with anti-CD3 mAb, whereas TPA/ionomycin has no or little effect. After 48 hours, this upregulation with anti-CD3 mAb is particularly strong, and a slight but not significant increase can be observed for samples treated with TPA/ionomycin, probably as a result of the accumulation of the protein in the supernatant. This behavior is similar in all 3 groups, suggesting that the capability to upregulate and produce Fas is well preserved with age. When it is considered that Fas is also involved in cell activation, this observation is in agreement with the preserved capability of lymphocytes from elderly subjects to proliferate even at extreme age in the presence of well-defined mitogenic stimuli.[4,5]

In regard to FasL, the upregulation of this gene by TPA/ionomycin is particularly efficient in lymphocytes from centenarians, in whom the levels of mRNA for FasL after stimulation become similar to those from young donors, despite the lower basal levels. From this point of view, centenarians seem to be characterized by a peculiar elasticity in the functionality of FasL, which probably is maintained at very low levels in quiescent lymphocytes to avoid inadequate cell death but can be strongly activated when required. Thus, centenarians are able to avoid excessive lymphocyte apoptosis in basal conditions but have an extremely efficient Fas/FasL apoptotic pathway, inducible after stimulation. This aspect can probably contribute to the well-preserved functionality of the immune system of these exceptional individuals and likely confers an advantage for longevity.

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