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Sex and parity modulate cytokine production during murine ageing

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SUMMARY

We have previously shown that physiological hormone differences related to pregnancy or sex affect the age-related distribution of mononuclear cell populations during murine ageing. To determine whether such changes are involved in the age-related changes in functions of T cells, we examined the secretion of major T cell immunoregulatory cytokines (IL-2, IL-4, interferon-gamma (IFN-γ), IL-3, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF)) of *in vitro* concanavalin A-activated spleen cells of C57Bl/6 mice. The study included multiparous and virgin females and males at 2, 8, 15 and 23 months of age. Short-term effects of parity (8 months) were evidenced by the decrease of IFN-γ and the preserved IL-2 production in multiparous females (8 months), while IFN-γ was unchanged and IL-2 decreased in virgin mice. The increase in IL-4 production appeared earlier in multiparous females (15 months) than in virgin mice (23 months). The increase in IL-4/IFN-γ and IL-4/IL-2 ratios at 8 and 15 months, respectively, in multiparous females, suggests that pregnancy modifies the Th1/Th2 equilibrium. In late adulthood (15 months), IL-6 and GM-CSF production was higher in multiparous females than in virgin males or females. Sex differences were also noticed: IFN-γ secretion capacity was lower in males than in females during ageing. This study underlines that the onset, magnitude and kinetics of the age-related changes in cytokine production are parity- and sex-dependent. These changes probably influence the incidence of age-related diseases and may explain the greater longevity of females.

Keywords mice ageing sex pregnancy cytokine

INTRODUCTION

Ageing is accompanied by functional disorders affecting both humoral and T cell-mediated immunity and is generally linked with an increased suceptibility to infections, malignancies and autoimmune diseases [1,2]. Changes in T cell functions in aged-related immune dysregulation are often associated with modified cytokine production [3]. The latter is involved in decreased helper functions such as the development of DTH responses, cytotoxic T cell precursors and antibody production by B cells [4]. It is well established that the balance of cytokines produced by functionally distinct T cell subsets modulates the immune response. Th1, Th2 and Th0 cells were first identified by *in vitro* analysis of murine T helper cell clones, but similar subsets exist *in vivo* in mice and humans [5–7]. Th1-like cells secrete IL-2, interferon-gamma (IFN-γ) and lymphotoxin, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. The development of Th1 or Th2-like response is influenced by antagonistic effects of cytokines secreted by each subset [7,8]. T cell IL-2 production declines with ageing [9], while that of IL-4, IFN-γ and IL-10 increases [10–15]. This may be due to changes in T cell equilibrium. In mice, the number of $CD44^{high}$ memory T cells increases with age and these cells produce more IL-4 and IFN-γ than $CD44^{low}$ naive T cells [13,14, 16–19]. The overproduction of certain cytokines may influence the age-related Th subset changes and probably contributes to the development of modified immune responses with ageing.

The role of sex hormones on immune reactivity is obvious both in experimental animals and humans [20]. During pregnancy, higher sex hormone levels influence maternal immune reactivity and profound changes such as the involution of the thymus and the increase in suppressive cells affect T and B lymphopoiesis [21,22]. Before our previous report [23], all studies on the murine ageing process had been conducted on virgin mice. We have shown that physiological influences related to sex hormonal differences affect age-related changes of immune cells. We reported that parity

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induces a delayed increase in memory $CD4^+$ $CD44^{high}$ cells early in life (8 months), whereas the memory/naive $CD4^+$ ($CD44^{high}$/$CD44^{low}$) ratio was higher in 23-month-old parous mice compared with their virgin counterparts. We equally reported that Mac-1^+ cell level and the memory/naive ratio were lower in males during ageing [23]. In this study, we analysed profiles of cytokine production during ageing related to the known parity- and/or sex-dependent qualitative and quantitative changes of immunocompetent cells during ageing. We investigated IL-2, IL-4, IFN-γ, IL-3, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) production by *in vitro* activated spleen cells of virgin and multiparous females and male C57Bl/6 mice at 2, 8, 15 and 23 months. Our results show distinct changes of cytokine production that may be due to two Th cell subpopulation changes related to pregnancies or sex differences during ageing.

MATERIALS AND METHODS

Animals

Male, virgin and multiparous female C57Bl/6JIco mice were purchased from IFFA Credo (L'Arbresle, France) at the age of 8, 15 and 23 months; 2-month-old males and females were used as

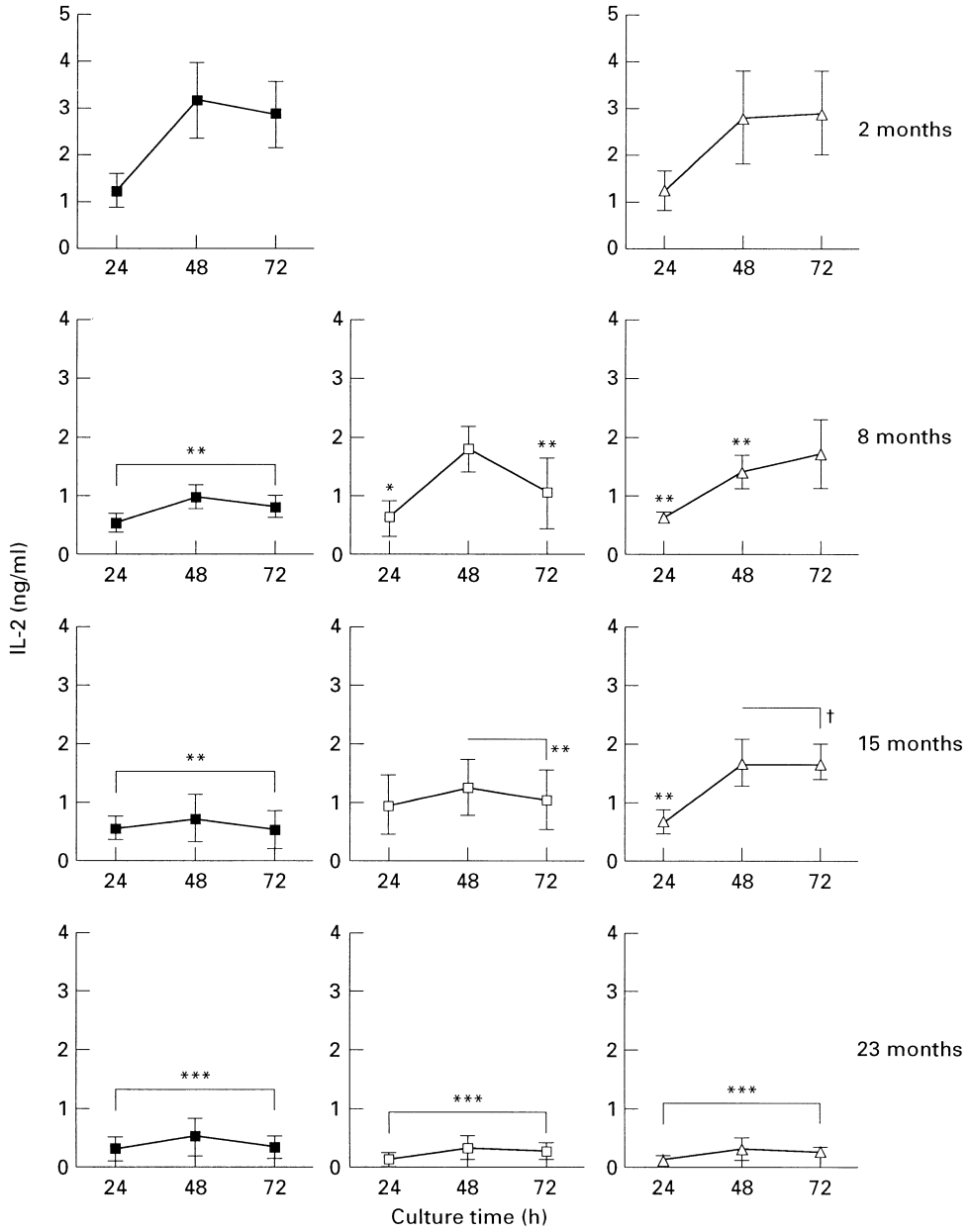

Fig. 1. Influence of sex or parity on IL-2 production by concanavalin A (Con A)-stimulated spleen cells of multiparous (□), virgin female (■) and male mice (△). Data are expressed as mean values ± s.e.m. from individual mice. Statistical analysis showed a significant age effect: †$P < 0{\cdot}02$; *$P < 0{\cdot}01$; **$P < 0{\cdot}001$; ***$P < 0{\cdot}0001$ between young and aged mice at the time of culture. At 8 months, IL-2 production was lower in virgin females than in multiparous mice and males ($P < 0{\cdot}02$) at 48 h and 72 h of culture, respectively; at 15 months the IL-2 level was lower in virgin females than in males ($P < 0{\cdot}02$) at 72 h.

controls in each experiment. Females were mated when they were between 10 and 25 weeks old and then separated from syngeneic males at the age of 5 months following five controlled gestations as previously described [23]. Three separate experiments including males, virgin and multiparous females and 2-month-old males and females as controls were used with a total of 24, 12, six and six animals, respectively, at 2, 8, 15 and 23 months of age. All mice were maintained by the producer until use, in specific pathogen-free colonies according to the specifications of the National Institute of Ageing. Mice were killed 8 to 12 days after receipt. Mice with evidence of pathology process were discarded.

Cell preparation and cytokine production

Individual samples of spleen cells were collected as previously described [23]. Cells were suspended in RPMI 1640 medium (GIBCO-BRL, Paisley, UK) supplemented with antibiotics (penicillin 100 U/ml; streptomycin 100 μg/ml), L-glutamine 2 mM (Biowhittaker, Walkersville, MD), 2-mercaptoethanol 0·2 μM (Sigma-Aldrich, Saint Quentin Fallauier, France) and 5% fetal calf serum (FCS; GIBCO-BRL). Cells ($1{\cdot}5 \times 10^6$/ml) were cultured in 96-well flat-bottomed microtitre plates with optimal concentration of concanavalin A (Con A; 2·5 μg/ml) (Sigma Aldrich) at 37°C with a 5% CO_2 humidified atmosphere during 72 h. Supernatants (SN) were collected after 24, 48 and 72 h from separate cultures. After centrifugation, they were kept frozen (−20°C) until tested for cytokine activity. Control samples of unstimulated spleen cells were simultaneously prepared and tested.

Cytokine determination

IL-2, IL-4, IFN-γ, IL-3, IL-6 and GM-CSF were detected by bioassays. Serial two-fold dilutions of SN, starting at 1:2 or 1:10, were cultured with appropriate cell lines as briefly described below. Specificity of all bioassays was confirmed by neutralizing the response with specific MoAbs. Each cell line response was assessed using recombinant cytokines IL-1, IL-2, IL-4, IFN-γ, IL-3, IL-6, tumour necrosis factor (TNF-α) and GM-CSF (R&D Systems, Abingdon, UK).

IL-2 was evaluated by measuring proliferation of the CTLL2-G4 line [24]. Cells (1×10^4/well) were incubated with supernatants for 32 h. Each well was then pulsed with 0·5 μCi ^{3}H-thymidine (specific activity 2 Ci/mmol; Amersham International, Aylesbury, UK) for a further 16 h. Cells were then harvested using a semi-automated collector (Inotech, Lansing, MI) and scintillation counting was carried out with a β counter (Wallac-Oy, Turku, Finland). In our experimental conditions, CTLL2-G4 cell line responded both to IL-2 and IL-4. This cell line responded to IL-4 concentrations which were 20 000 above those found in the maximal SN IL-4 content. Anti-IL-4 was therefore used in some experiments to test whether the SN IL-4 content influenced the results.

IL-4 was measured with the CT4S cell line as previously described [25]. Cells (1×10^4/well) were incubated with SN for 72 h and pulsed with 1 μCi/well of ^{3}H-thymidine (specific activity 5 Ci/mmol) during the last 18 h. The CT4S cell line proliferated with 15 ng/ml of rIL-2, exceeding concentrations found in our SN (maximum 4 ng/ml).

IFN-γ assay. IFN-γ levels in SN were titrated for their ability to inhibit the cytopathic effect of vesicular stomatitis virus (VSV) on a mouse fibroblast line (L929) as previously described [26]. L929 cells (2×10^4/well) were seeded in 96-well microtitre plates with 100 μl of Eagle's minimal essential medium (GIBCO-BRL) supplemented with 100 nmol/*l* L-glutamine and 5% FCS to give single-layer confluent cell growth. After 24 h of incubation, dilutions of SN or known concentrations of rIFN-γ (control) were added to cells. Plates were drained after 24 h of culture, and 100 plaque-forming units (PFU) of VSV were added to a complete medium supplemented with 2% FCS. Titres were expressed in laboratory units as the reciprocal of the dilution giving 50% of the cytopathic effect. In this experiment, one international unit of IFN-γ represented one laboratory unit. The lowest level of IFN-γ detection was 2 U/ml.

IL-6 activity was assessed with the IL-6-dependent B9 cell line [27]. Cells (1×10^4/well) were incubated with SN. After 60 h of culture, cells were labelled with 1 μCi/well of ^{3}H-thymidine (specific activity 5 Ci/mmol) for 6 h.

IL-3 and GM-CSF activities were evaluated with FDC-P2 and FDC-P1 cell lines, respectively, as previously described [28,29]. Cells were incubated for 24 h and pulsed with 1 μCi/well of ^{3}H-thymidine (5 Ci/mmol) 16 h before the end of culture time. GM-CSF activity was assessed on the FDC-P1 cell line in the presence of excess amounts of anti-IL-3 MoAb (1 μg/well), since FDC-P1 cells are sensitive to IL-3 as well as GM-CSF [27]. This cell line is not sensitive to the cytokines tested.

In IL-2, IL-3, IL-4, IL-6 and GM-CSF bioassays, data were quantified from standard curves using specific recombinant cytokines and calculated from reciprocal dilution that gave half maximal activity. Under our culture conditions, the lowest detection limit was 5 pg/ml for IL-2, IL-3 and IL-6, 1 pg/ml for IL-4 and 10 pg/ml for IFN-γ and GM-CSF. Each cell line bioactivity was completely neutralized by each specific anti-IL MoAb.

Statistical analysis

Data were analysed using the non-parametric Mann–Whitney *U*-test and ANOVA based on the SAS statistical package [30]. For each parameter, age effect was analysed by comparing each age group with young adults of the same sex. Parity or sex effect were evaluated by comparing results of mice of similar ages but of different sex or parous status.

RESULTS

Age-related changes in IL-2 production

IL-2 production in supernatants of Con A-activated spleen cells was evaluated after 1, 2 and 3 days of culture. In young adults of both sexes, IL-2 production increased between days 1 and 2 and remained unchanged at day 3 (Fig. 1). IL-2 production in 8-month-old virgin female mice significantly decreased ($P < 0{\cdot}001$) over all culture times and represented approximatively 30% of the level observed in young females. At 8 months, a significant parity and sex effect ($P < 0{\cdot}02$) was observed in multiparous females and males with IL-2 levels not significantly different from those in young mice. A sex effect was observed at 15 months. Virgin females presented the lowest level of IL-2 compared with males ($P < 0{\cdot}02$). IL-2 levels were lowest in advanced age (23 months) males and multiparous females ($P < 0{\cdot}0001$) compared with those of middle age (15 months). No IL-2 was detected in SN of any age group in the absence of Con A.

IL-4 production and IL-4/IL-2 ratio with ageing

Data are presented at day 3 of culture supernatants when IL-4 peaks in mice, according to previous studies [25]. IL-4 production significantly increased in 23-month-old groups compared with young adults ($P < 0{\cdot}001$) (Fig. 2a). This age-related change in

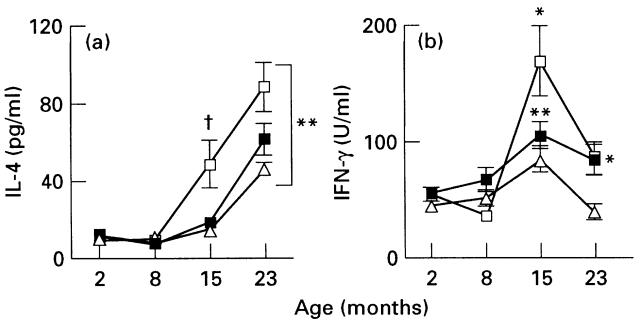

Fig. 2. Age-related changes of IL-4 (a) and IFN-γ (b) production by concanavalin A (Con A)-stimulated spleen cells of multiparous (□), virgin female (■) and male mice (△). Data are expressed as mean values ± s.e.m. obtained from individual mice after 72 h of culture. Statistical analysis showed a significant age effect: †$P < 0{\cdot}02$; *$P < 0{\cdot}01$; **$P < 0{\cdot}001$ between young (2-month-old) and aged (8-, 15- and 23-month-old) mice. IL-4 production was higher in multiparous females than in other groups at 15 months ($P < 0{\cdot}02$) and at 23 months *versus* males ($P < 0{\cdot}005$), and virgin females ($P < 0{\cdot}04$). The IFN-γ level was higher in multiparous females than in other groups at 15 months ($P < 0{\cdot}01$). At 23 months, males showed the lowest IFN-γ level ($P < 0{\cdot}01$).

IL-4 production appeared earlier on, in 15-month-old multiparous females compared with young adults ($P \leq 0{\cdot}02$). IL-4 levels were higher in multiparous females than in virgin females ($P < 0{\cdot}02$) and males ($P < 0{\cdot}005$) at 15 and 23 months (Fig. 2a). Related to changes in IL-4 and IL-2 secretion, the IL-4/IL-2 ratio obtained from individual mice significantly increased from 15 months in the female groups ($P < 0{\cdot}005$) (Fig. 3a) and continued to increase thereafter. This ratio was more pronounced in all 23-month-old groups ($P < 0{\cdot}005$). A significant parity effect was observed with a higher IL-4/IL-2 ratio in multiparous females ($P < 0{\cdot}005$) than in virgin females or males at the age of 15 and 23 months.

IFN-γ production and IL-4/IFN-γ ratio with ageing

According to previous studies in mice, peak IFN-γ production is obtained on day 3 of culture [26]. Female groups exhibited a significant increase of IFN-γ production (Fig. 2b) at 15 months ($P < 0{\cdot}01$), which then fell at 23 months ($P < 0{\cdot}001$) yet remained higher than young adult levels. In contrast, no males showed

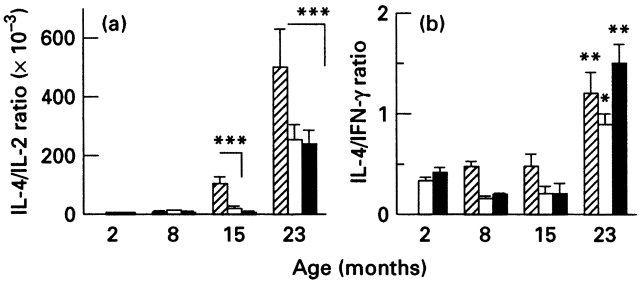

Fig. 3. Influence of sex and parity on the variation of IL-4/IL-2 (a) and IL-4/IFN-γ (b) ratio by stimulated spleen cells during ageing. Data are expressed as mean values ± s.e.m. from individual mice, and obtained on day 3 of culture. Statistical analysis showed a significant age effect between young and aged mice of the same sex (*$P < 0{\cdot}05$; **$P < 0{\cdot}001$; ***$P < 0{\cdot}005$). The IL-4/IL-2 ratio was higher in multiparous (▨) than in virgin females (□) and males (■) at 15 months ($P < 0{\cdot}005$). At 8 months, the IL-4/IFN-γ ratio was highest in multiparous groups ($P < 0{\cdot}05$).

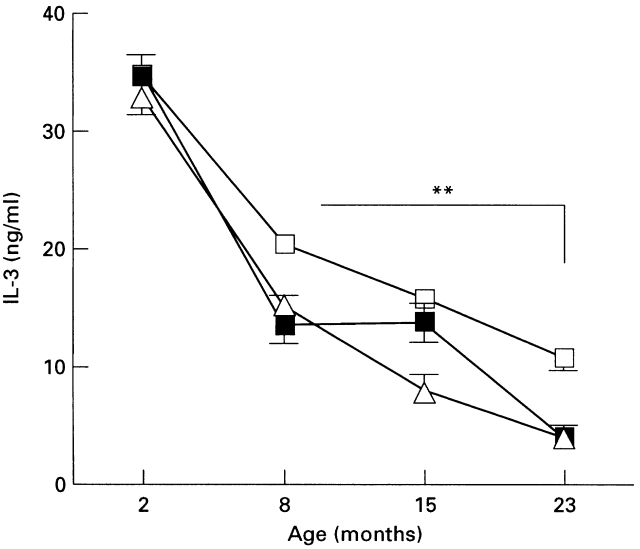

Fig. 4. Age-related changes in IL-3 production by concanavalin A (Con A)-stimulated spleen cells of multiparous (□), virgin females (■) and male mice (△). Data are presented as mean values ± s.e.m. obtained on day 2 of culture. Statistical analysis showed a significant age effect between young and aged mice of the same sex (**$P < 0{\cdot}001$). IL-3 production was significantly higher in multiparous ($P < 0{\cdot}02$) than in other groups at 23 months.

significant differences in all age groups. Consequently, a sex effect was observed between male and female groups at 23 months ($P < 0{\cdot}01$). A significant parity effect was observed at 15 months: IFN-γ levels were highest in multiparous females ($P < 0{\cdot}01$). The IL-4/IFN-γ ratio significantly increased in the oldest groups (Fig. 3b). A parity effect was observed in the IL-4/IFN-γ ratio which was significantly higher in multiparous females ($P < 0{\cdot}05$) due to lower IFN-γ and higher IL-4 release at 8 and 15 months, respectively.

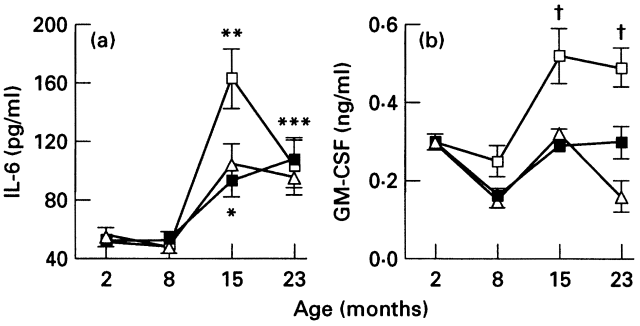

Fig. 5. Age-related changes in IL-6 (a) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (b) production by stimulated spleen cells of multiparous (□), virgin female (■) and male mice (△). Data are presented as mean values ± s.e.m. obtained on day 1 (IL-6) and day 3 (GM-CSF) of culture. Statistical analysis showed a significant age effect between young and aged mice of the same sex (†$P < 0{\cdot}02$; *$P < 0{\cdot}01$; **$P < 0{\cdot}005$; ***$P < 0{\cdot}001$). Multiparous females showed higher GM-CSF levels ($P < 0{\cdot}02$) at 15 and 23 months and IL-6 ($P < 0{\cdot}01$) production at 15 months than other groups.

Age-related changes in IL-3 production

Age-related changes in IL-3 production obtained after 48 h of activation are presented in Fig. 4. Similar results were obtained on day 3 (data not shown). IL-3 levels were low after 24 h (<5 ng/ml) with no significant difference between young and old mice. IL-3 secretion (at 48 h) significantly decreased ($P < 0{\cdot}001$) between 2 and 23 months in all groups. The decrease in IL-3 production with ageing was lowest in the multiparous females in the 23-month-old group ($P < 0{\cdot}02$).

IL-6 and GM-CSF production during ageing

After 24 h of activation, IL-6 release (Fig. 5a) significantly increased with age at 15 months in multiparous ($P < 0{\cdot}005$) and virgin females and males ($P < 0{\cdot}01$) compared with younger mice. Thereafter, IL-6 production fell in all groups at 23 months, but remained higher than in young adults ($P < 0{\cdot}001$). No significant differences were obtained after 48 h and 72 h of culture between young and old groups (data not shown). At 15 months, the IL-6 increase was significantly higher in parous mice ($P < 0{\cdot}01$).

GM-CSF production was unchanged in virgin females and males during ageing. In contrast, GM-CSF secretion (Fig. 5b) increased in multiparous mice at 15 months compared with young adults ($P < 0{\cdot}02$) and levelled off thereafter. GM-CSF production was highest in 15 and 23-month-old multiparous females ($P < 0{\cdot}02$).

DISCUSSION

This study demonstrated that physiological hormonal differences related to pregnancy or sex influenced the patterns of cytokine production during murine ageing. Parity effects are related to: (i) a lower decrease of IL-2 secretion at 8 and 15 months of age and IL-3 production at 23 months in parous females than in virgin counterparts; (ii) an increase in IL-4, IFN-γ, IL-6 and GM-CSF production and IL-4/IL-2 ratio in middle-aged parous mice compared with virgin females. Sex differences were observed only in the smaller decrease of IL-2 during adulthood (8–15 months) and lower IFN-γ production in males during ageing.

Our first investigation concerned the effects of sex difference on cytokine production in virgins. We found a diminished IL-2 production early in life (8 months), while the large majority of reports showed that such changes occurred later in life (12–28 months) [9]. It is well established that ageing is correlated to impaired production of IL-2 by *in vitro* stimulated T cells in rodents and humans [3,4]. In adulthood (8–15 months), males showed higher IL-2 levels compared with virgin females, nevertheless IL-2 secretion decreased to a similar extent in both sexes at an advanced age. An early decrease of IL-3 production in 8-month-old virgins was also observed. Studies showed conflicting results about IL-3 production which increased [31] or decreased [11,32] with age. Our results showed a parallel loss in IL-2 and IL-3 production that suggests a decline in T cell responsiveness earlier than in other reports [8,31]. In a previous study, we reported that the switch of naive ($CD44^{low}$) to memory ($CD44^{high}$) T cells occurred early in life (8 months) in males and virgin females [23,33]. Both phenotypic changes and IL-2 and IL-3 decreases seem to be due to increased memory T cells which produce less IL-2 [16,17] and/or to the reduced secretory capacity of old naive T cells [34]. Our analysis supports these hypotheses, pointing out that changes may occur early in adulthood as shown for the naive/memory switch in human T cells [35].

An increase in IL-4 production was found for virgins only in late life (23 months). Our results confirmed previous findings obtained at 20–30 months, on activated total spleen cells or isolated $CD4^+$ T cells [12,14,18]. The capacity to secrete IL-4 is predominant in memory $CD4^+$effector populations [17,36]. We previously reported a lower memory/naive ($CD44^{high}/CD44^{low}$) ratio in $CD4^+$ cells in males than in females [23]. The age-related increase of IL-4/IL-2 ratio (a reflection of Th2/Th1 responses) appeared earlier in females than in males and was mainly due to an increase in IL-4 production, and also could be related to the different timing of the memory/naive ratio [23]. These results suggest that a predominant Th2 activity occurred earlier in the menopausic period of females, and underline sex differences in immune responses during ageing.

With advancing age, IFN-γ secretion remained steady in males, while it increased in virgin females at 15 and 23 months. The high IFN-γ response observed in old females accords with previous studies carried out with *in vitro* Con A-activated spleen cells [37]. In contrast, increased IFN-γ production by isolated $CD4^+$ [12,14], $CD8^+$ [13] or pure T cells [38] whithout adherent cells was reported in aged male mice. Our results were obtained on total spleen cells, including macrophages. The sex-related differences in IFN-γ production during ageing may be linked to the lowest level of macrophages in aged males previously observed [23]. Furthermore, it has also been demonstrated that macrophages selectively influence the polarized response towards the preferential development of a Th-2-type response [39] and could explain the lower Th2/Th1 responses discussed above. Impaired production of IFN-γ could also be related to $CD8^+$ T cells involved in cytotoxic response. The failure of such a response may result in ongoing disease. In experimental viral infections, impaired capacity to produce IFN-γ in young male mice has been shown and could explain the predominance of myocarditis disease in men [40]. Our study underlines that physiological sex hormonal differences affect age-related changes of cytokine production and may contribute to, or exacerbate, the more frequent development of autoimmune responses in women [41] and the earlier mortality in men.

The second part of our study investigated the influence of transient hormonal changes occurring during pregnancies on immune ageing. Parity has a short-term effect on immune responses, as shown by a lower decrease in IL-2 and IL-3 production and a lower IFN-γ level in parous compared with virgin females at 8 months. Pregnancy profoundly depresses the maternal immune system [21,22]. Thymus atrophy is followed by a rebound effect in T cell differentiation which induces a higher emigration of naive $CD4^+$ cells [42]. We have shown that pregnancies delay the increase of memory $CD4^+$ subsets ($CD44^{high+}$) early in life (8 months) [23]. Such a delay may explain the lowest IFN-γ level and highest IL-2 level in 8-month-old multiparous females. Naive cells secrete more IL-2 and less IFN-γ than memory T cells [16,36]. We postulate that phenotypic changes observed previously in multiparous females might be linked to these functional changes.

Moreover, a long-term effect of parity was observed, including increased production of IL-4, IFN-γ, IL-6 and GM-CSF in 15-month-old multiparous mice. The increased numbers of Mac-1^+ macrophages in 15-month-old multiparous mice of 8, 15 and 23 months in our previous report [23] and the stimulatory effect of persistent trophoblastic cells maintained for a long time after delivery (Dr G. Chaouat, personal communication) could contribute to these

different patterns of cytokine production, directly through macrophage cytokine release or indirectly through macrophage activity on Th subsets. According to Duncan and Swain [39], antigen-presenting cells might influence Th subset development, particularly under conditions of prolonged or chronic stimulation, in an autocrine fashion, and exogenous cytokines such as IL-4 and IFN-γ have a predominant effect in this way. The increase in IL-4 and IL-6 production and the IL-4/IL-2 ratio of aged multiparous mice suggest that pregnancies could induce a preferential development of Th2-like response in middle-age. We might wonder whether the observed changes are related long-term stimulation of the immune system after pregnancy, as suggested by Chaouat (see above).

IL-6 and GM-CSF overproduction observed in multiparous females (15 months and 23 months) could be explained by the elevated number of Mac-1$^+$macrophages in parous mice [23]. It is known that IL-6 and GM-CSF are produced by both Th2 cells and monocytes/macrophages [43]. Increased IL-6 production in males and virgin females observed in late life is in accordance with other reports on mice [44,45] and the elderly [46,47]. The highest production of IL-3 (at 8–23 months) and GM-CSF (at 15–23 months) observed in multiparous females concerns colony-stimulating factors (CSF) involved in myelopoiesis. We believed that this increase in CSF production may explain the highest Mac-1 cell levels previously reported in multiparous females [23]. No comparative studies have been conducted on the influence of parity during the immune ageing process in mice or humans. Pregnancy generally aggravates ongoing autoimmune diseases, but in some pathologies, transient improvement may occur. Aggravation may be related to the effect on Th1/Th2 cytokine equilibrium reported here. These observations might also be important in the critical Th1/Th2 switch in HIV disease, which also induces a more important autoimmune phenomenon in parous females [48]. Interactions between the reproductive and maternal immune system could influence immune competence during ageing. The overproduction of certain immunoregulatory cytokines could either suppress or amplify immune responses in ageing.

Our study underlines that phenotypic and functional changes of immunocompetent cells begin earlier in adulthood than previously reported. The different patterns of cytokine production observed in this study emphasize that physiological hormonal differences related to sex or pregnancy are involved in the age-related immune dysregulation. More information on cytokine interregulation after pregnancy may allow us to design strategies more suitable for therapy of infections and autoimmune diseases, vaccination or immunomodulation.

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