Enhanced natural killer activity and production of pro-inflammatory cytokines in mice selected for high acute inflammatory response (AIRmax)

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Summary

Strains of mice with maximal and minimal acute inflammatory responsiveness (AIRmax and AIRmin, respectively) were developed through selective breeding based on their high- or low-acute inflammatory responsiveness. Previous reports have shown that AIRmax mice are more resistant to the development of a variety of tumours than AIRmin mice, including spontaneous metastasis of murine melanoma. Natural killer activity is involved in immunosurveillance against tumour development, so we analysed the number and activity of natural killer cells ($CD49b^+$), T-lymphocyte subsets and *in vitro* cytokine production by spleen cells of normal AIRmax and AIRmin mice. Analysis of lymphocyte subsets by flow cytometry showed that AIRmax mice had a higher relative number of $CD49b^+$ cells than AIRmin mice, as well as cytolytic activity against Yac.1 target cells. The number of $CD3^+$ $CD8^+$ cells was also higher in AIRmax mice. These findings were associated with the ability of spleen cells from AIRmax mice *in vitro* to produce higher levels of the pro-inflammatory cytokines tumour necrosis factor-α, interleukin-12p40 and interferon-γ but not the anti-inflammatory interleukin-10. Taken together, our data suggest that the selective breeding to achieve the AIRmax and AIRmin strains was able to polarize the genes associated with cytotoxic activity, which can be responsible for the antitumour resistance observed in AIRmax mice.

Keywords: cytotoxicity; immunogenetics; inflammation; innate immunity; natural killer cells

Introduction

Acute inflammatory responsiveness (AIR) strains of mice were developed by bidirectional phenotypic selection on the basis of their acute inflammatory response.[1] The bidirectional selective breedings were initiated from a highly polymorphic population (F_0) and selected based on the degree of local AIR measured by the responsiveness to polyacrylamide beads, a chemically inert and non-immunogenic substance.[1–3] At the selection limit, the mouse lines AIRmax and AIRmin, named for their high and low responses respectively, differed 20-fold in the number of infiltrated leucocytes and 2·5-fold in protein concentration in the 24-hr subcutaneous inflammatory exudates.[4] This large difference between the infiltrate of the cell lines reflects the altered granulopoietic response of bone marrow to haematopoietic factors, as well as the ability of the granulocytes found in the exudates from AIRmax mice to resist apoptosis.[5] Furthermore, high local production of chemotactic factors by infiltrated or resident AIRmax cells also contributes to the phenotypic difference between the lines.[5]

Previous studies have shown that AIRmax mice are more resistant to the development of chemical skin carcinogenesis induced by 9,10-dimethyl-1,2-benzantracene and 12-*O*-tetradecanoyl-phorbol-13 acetate.[2] Biozzi *et al.*[2]

Abbreviations: AIRmax, mice with maximal acute inflammatory responsiveness; AIRmin, mice with minimal acute inflammatory responsiveness; CTL, cytotoxic T lymphocyte; FITC, fluorescein isothiocyanate; IFN-γ, interferon-γ; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; PE, phycoerythrin; SAC, *Staphylococcus aureus* Cowan I; Th1, T helper type 1; TNF-α, tumour necrosis factor-α.

observed that AIRmax animals showed lower incidence and multiplicity of neoplasia than AIRmin animals. Moreover, AIRmax mice were more resistant to the development of metastases of murine (B16F10) and human (SKmel-28) melanoma.[3] AIRmin mice were also more susceptible to lung carcinogenesis induced by urethane, showing 40 times more tumour foci than AIRmax mice;[4,6] the authors indicated that this susceptibility was associated with the segregation of genes that control the susceptibility to lung carcinoma (PAS-1).

Although inflammatory cytokines such as tumour necrosis factor-α (TNF-α) are able to destroy some tumour cells, these findings contrast with the concept that the inflammatory response is associated with cancer development and that a chronic response can represent a promoter factor for carcinogenesis[7,8] because the association of DNA damage with growth factors and the process of angiogenesis at the inflammatory site enhance the development and growth of neoplastic cells.[9–11] Considering this paradigm, it is reasonable to speculate not only that the higher resistance to tumour growth shown by AIRmax mice is the result of the inflammatory response but also that other antitumoral effector mechanisms may be involved. In fact, there is evidence that specific cytotoxic T lymphocytes (CTLs),[9] natural killer (NK) cells[12] and a variety of cytokines[13] constitute the main antitumour weapons of the host immune system. Since the selective process for obtaining AIRmax and AIRmin strains appears not to interfere with specific cellular or humoral immune responsiveness,[1,2,14] it is possible to assume that the effect on non-specific NK activity is associated with differences in antitumour resistance.

Natural killer cells are involved in antitumour and antiviral responses because of their ability to destroy target cells by a major histocompatibility complex (MHC) -unrestricted mechanism[15,16] without previous stimulation of immune response.[9,17,18] The NK cells are heterogeneous in their cell-surface phenotype, proliferative capacity and function.[19,20] Their function is tightly regulated by a delicate balance of inhibitory and activating signals delivered through a diverse array of cell-surface receptors, as well as mediated by pro-inflammatory cytokines.[20] Destruction of target cells involves the release of cytoplasmic granule toxins, predominantly perforin and granzymes, which requires direct contact between the effector and target cells and activates cell-death pathways through the activation or not of apoptotic cysteine proteases (caspases).[20,21] Natural killer cells are also able to induce apoptosis of target cells through the interaction of Fas ligand with Fas, which results in classical caspase-dependent apoptosis, expressed by a variety of tumour cells, virus-infected cells and self-reactive lymphoid cells.[20,22–24] Besides their effector cytotoxic activity, NK cells are also involved in the organization of the specific immune response by secreting several cytokines such as interferon-γ (IFN-γ) and TNF-α,[18,25,26] which are able to drive the immune response to the T helper type 1 (Th1) feature, stimulating both $CD8^+$ cytotoxic cells and macrophage activation.

Aiming to test the hypothesis that the selective process to obtain the strains of mice with intense or weak inflammatory response (AIRmax/AIRmin) results in the segregation of genes associated with NK activity, this study analysed the number and cytotoxic activity of NK cells of normal AIRmax and AIRmin animals and the ability of their spleen cells to produce the cytokines TNF-α, IFN-γ, interleukin-12 (IL-12) and IL-10.

Materials and methods

Animals

Twelve-week-old male and female mice of the AIRmax and AIRmin strains were obtained from the Laboratory of Immunogenetics of the Institute Butantan, São Paulo, Brazil. After 2 weeks of acclimatization in the Animal House of the Department of Pathology, Faculdade de Medicina de Botucatu, UNESP, the animals were randomly distributed into polypropylene cages ($n = 10$), with commercial feed and water *ad libitum* at 22° under 12 : 12 hr dark : light cycles.

The animals were anaesthetized intraperitoneally with sodium pentobarbital and the spleen was removed fro each mouse to obtain the lymphocyte suspension. All the procedures involving animals were performed in accordance with the Brazilian College for Animal Experimentation (COBEA) and were approved by the Ethical Committee for Animal Experimentation at the Faculdade de Medicina de Botucatu, UNESP (process no. 440).

Natural killer activity

Spleen cell suspensions were obtained by teasing these organs on a sterile fine nylon screen into RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (Cultilab, Campinas-SP, Brazil), 20 mM HEPES (Sigma-Aldrich, St. Louis, MO), 0·2% sodium bicarbonate, 2×10^{-5} M mercaptoethanol and 40 IU gentamicin (complete culture medium), and the NK activity of non-adherent cells was assayed according to Kaneno *et al.*[27]

Monoclonal antibodies for flow cytometry

Quantification of NK cells ($CD49b^+$) was performed using fluorochrome-labelled monoclonal antibodies from BD Pharmingen (San Diego, CA): fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD49b/Pan-NK, phycoerythrin (PE)-conjugated hamster anti-mouse CD3ε-chain, FITC-conjugated rat anti-mouse CD4(L3T4), FITC-conjugated rat anti-mouse CD8a(Ly-2), and their respective isotype controls.

Analysis of lymphocyte subsets

One of the spleen fragments was teased on a fine nylon screen to obtain a suspension of total spleen cells that was adjusted to 10^7 cells/ml. The cell suspension of each mouse was placed on a 'U'-bottomed 96-well microtitre plate (100 μl/well) and cultured for 10 min with 10 μl normal mouse serum (for blocking non-specific sites) following incubation with specific monoclonal antibodies or isotypic control, for 60 min. Analyses were performed in a FACSCalibur from Becton Dickinson Immunocytometry Systems (Becton Dickinson, Bioscience, San Jose, CA), in the Laboratory of Flow Cytometry of the Hemocentro, Faculdade de Medicina de Botucatu, UNESP. The spleen cells stained with FITC-labelled and PE-labelled antibodies were detected on FL1 and FL2, respectively. Fluorescence overlap was electronically calibrated using single-colour-stained standard beads (FITC or PE) and 10 000 events were acquired and stored for each analysis. Spleen cells were identified for gating by their characteristic appearance on a dot plot of forward scatter versus side scatter. Results were expressed as the percentage of positive cells within a gate.

Generation of culture supernatants for cytokine analysis

Spleen-cell suspensions were adjusted to 4×10^6 cells/ml in complete culture medium. Cells were distributed among 96-well flat-bottomed microculture plates (100 μl/well) and were cultured with concanavalin A (Sigma Aldrich; 3·5 μg/well) for 24 hr for the generation of IL-10 and IFN-γ, or with formolized *Staphylococcus aureus* Cowan I suspension (SAC; Sigma Aldrich; 1 : 5000) for 48 hr for the generation of TNF-α and IL-12. The supernatants of the cultures were collected and frozen at −80° for further cytokine quantification.

Analysis of TNF-α, IFN-γ, IL-12p40 and IL-10 through enzyme-linked immunosorbent assay

Cytokines in culture supernatants were quantified using enzyme-linked immunosorbent assays with reagents from R & D Systems (Minneapolis, MN) for TNF-α, IFN-γ and IL-10 or from BD Pharmingen (San Diego, CA) for IL-12p40, following the indications of each manufacturer. Briefly, 96-well flat-bottomed microtitre plates (Nunc Maxisorp, Roskilde, Denmark) were sensitized with the specific capture antibody and non-specific sites were blocked with a quench solution (1% heat-inactivated fetal bovine serum, 5% saccharosis, 0·05% NaN_3). After washing, the samples or recombinant cytokines (standard curve) were distributed among the wells, following incubation for 2 hr at room temperature. The plates were washed and anti-cytokine biotinylated antibody was added to the wells after 1 hr. Streptavidin-peroxidase was then added to the wells and the reactions were revealed with orthophenylene/H_2O_2 (R & D Systems) or tetramethylbenzidine/urea peroxide solution (BD Pharmingen). The reaction was read by spectrophotometry under 450 nm (LabSystems, Helsinki, Finland). Data from the standard curve were submitted to linear regression analysis and the results were expressed as pg/ml.

Statistical analysis

The comparison between the lineages and gender was accomplished through 2 × 2 factorial analysis of fully randomized data (SAS Software System V8) and differences were considered significant when $P < 0{\cdot}05$.

Results

Natural killer activity

The NK cytotoxic activity of non-adherent spleen cells was evaluated through the classic chromium-release-based cytotoxicity test. The effector : target cell ratio we chose (50 : 1) was based on previous pilot experiments using diverse ratios (1 : 100, 1 : 50 or 1 : 25, data not shown). As can be observed in Fig. 1, NK activity was higher in the AIRmax animals (male AIRmax 7·24 ± 0·72%; female AIRmax 11·64 ± 2·46%) than in AIRmin (male AIRmax 3·74 ± 0·57%; female AIRmin 5·16 ± 1·84%). Statistical analysis showed no influence of gender on NK activity.

Quantitative analysis of $CD49b^+$, $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ cells in the spleen

Analysis of cell subsets by flow cytometry showed that the number of spleen NK ($CD49b^+$) cells was significantly

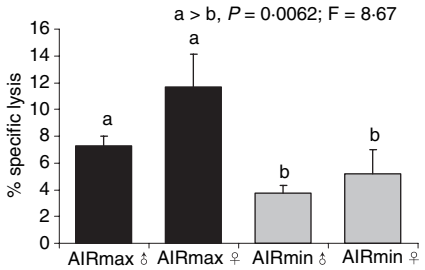

Figure 1. NK activity of effector spleen cells of male and female AIRmax and AIRmin animals ($n = 10$) was measured by 4-hr ^{51}Cr-release assay using labelled YAC-1 target cells (effector to target cell ratio was 50 : 1). Percentage cytotoxicity, as measured by specific ^{51}Cr-release, was calculated using the formula: 100 × (experimental c.p.m. − spontaneous c.p.m.)/(maximal c.p.m. − spontaneous c.p.m.), where c.p.m. are counts/min. Results are expressed as mean percentage and standard errors. Statistical analysis (2 × 2 factorial) showed that there was an effect of the strain background (AIRmax > AIRmin) but that gender did not affect NK activity (male = female).

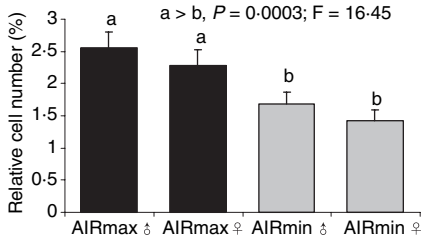

Figure 2. Flow cytometry analysis of relative numbers of spleen NK (CD49b) cells in AIRmax and AIRmin normal animals, male and female ($n = 10$). For quantification of NK cells ($CD49b^+$) FITC-conjugated rat anti-mouse CD49b/Pan-NK cell monoclonal antibody and FITC-conjugated rat IgM isotype controls (BD Pharmingen) were used. Analyses were performed in a FACSCalibur from Becton Dickinson Immunocytometry Systems. Results are expressed as percentage means and standard errors. Statistical analysis (2×2 factorial) showed a background effect (AIRmax > AIRmin) without gender influence (male = female for each strain).

higher in AIRmax mice (male AIRmax 2·56 ± 0·24%; female AIRmax 2·28 ± 0·24%) than in AIRmin (male AIRmin 1·68 ± 0·18%; female AIRmin 1·42 ± 0·17%). Statistical analyses demonstrated that this parameter was not influenced by gender (Fig. 2).

Both AIRmax and AIRmin mice presented similar numbers of $CD3^+$ spleen cells as displayed in Fig. 2. However, statistical analysis indicated that male mice had a significantly higher number of $CD3^+$ cells than their female counterparts ($P < 0·05$).

The number of $CD3^+$ $CD4^+$ cells in AIRmax mice (male AIRmax 19·58 ± 1·57%; female AIRmax 18·93 ± 3·52%) was not statistically different from that observed in AIRmin mice (male AIRmin 19·15 ± 1·48%; female AIRmin 18·02 ± 1·80%; Fig. 3). However, the number of AIRmax $CD3^+$ $CD8^+$ cells (male AIRmax 15·40 ± 0·95%; female AIRmax 9·08 ± 0·74%) was higher than that in AIRmin mice (male AIRmin 11·88 ± 1·10%; female AIRmin 8·47 ± 0·55%; Fig. 3). Statistical analyses also showed that male mice had more $CD3^+$ $CD8^+$ cells than female mice (Fig. 3).

In vitro production of cytokines by spleen cells

Since the generation and activity of NK cells is highly dependent on cytokine levels and constitutes the main source of early IFN-γ, we analysed the *in vitro* production of some cytokines by culturing spleen cells with appropriate stimuli.

Our results show that the levels of TNF-α produced by AIRmax mice (male AIRmax 346·93 ± 55·81 pg/ml; female AIRmax 306·96 ± 22·12 pg/ml) are higher than those produced by AIRmin (male AIRmin 99·11 ± 24·62 pg/ml; female AIRmin 73·58 ± 8·86 pg/ml; Fig. 4). This was also compatible with the levels of IFN-γ production that were also higher in AIRmax (male AIRmax

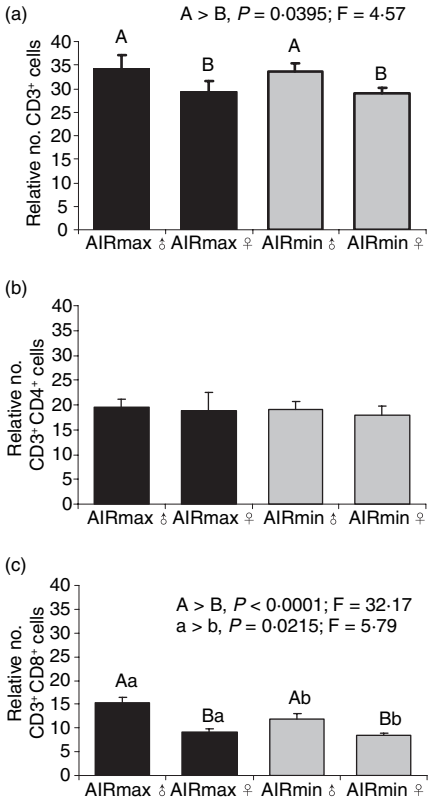

Figure 3. Flow cytometry analysis of relative number of spleen $CD3^+$ cells (a), $CD3^+$ $CD4^+$ cells (b) and $CD3^+$ $CD8^+$ cells (c) of male and female AIRmax and AIRmin normal animals ($n = 10$). For the $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ quantification PE-conjugated hamster anti-mouse CD3ε-chain, FITC-conjugated rat anti-mouse CD4(L3T4) and FITC-conjugated rat anti-mouse CD8a(Ly-2) monoclonal antibodies and their isotype controls (BD Pharmingen) was used. Analyses were performed in a FACSCalibur from Becton Dickinson Immunocytometry Systems. Results are expressed as percentage means and standard errors. Statistical analysis (2×2 factorial) showed that male mice have higher numbers of $CD3^+$ than female mice and that AIRmax mice have more $CD8^+$ cells than AIRmin mice.

747·04 ± 102·84 pg/ml; female AIRmax 895·84 ± 85·53 pg/ml) than in AIRmin (male AIRmin 609·35 ± 148·98 pg/ml; female AIRmin 520·19 ± 83·95 pg/ml; Fig. 4). AIRmax also produced more IL-12p40 than AIRmin (male AIRmax 306·64 ± 44·20 pg/ml; female AIRmax 207·20 ± 35·99 pg/ml; male AIRmin 100·10 ± 28·18 pg/ml; female AIRmin 99·52 ± 24·77 pg/ml; Fig. 4).

In vitro production of IL-10 was also evaluated (Fig. 4) but no significant differences were observed between the two strains (male AIRmax 1238·49 ± 95·06 pg/ml; female AIRmax 1100·60 ± 64·91 pg/ml; male AIRmin 1285·66 ± 103·18 pg/ml; female AIRmin 1205·30 ± 51·94 pg/ml).

Discussion

Previous studies have shown that mice selected for maximum acute inflammatory response (AIRmax) are more

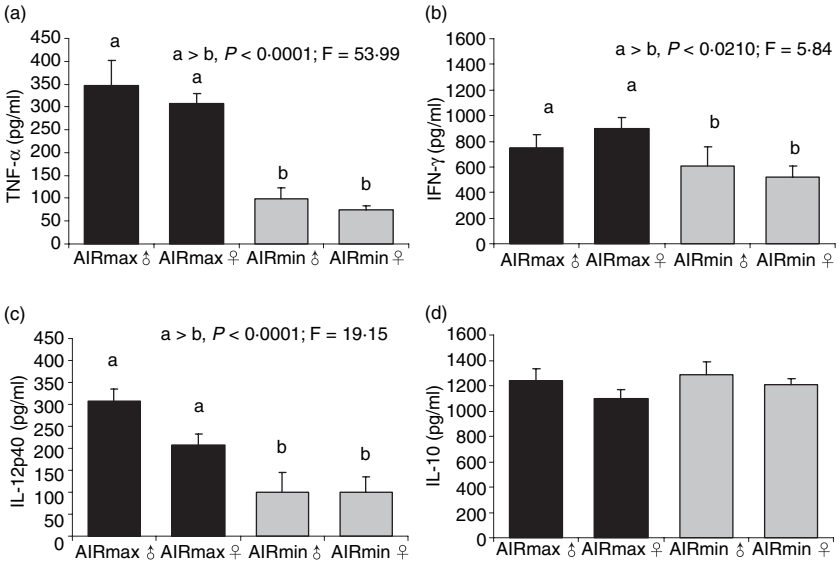

Figure 4. Cytokine production in the supernatants of spleen cell cultures from male and female AIRmax and AIRmin normal animals ($n = 10$). Quantifications were performed by enzyme-linked immunosorbent assay using reagents from R & D Systems for TNF-α, IFN-γ and IL-10 and BD Pharmingen for IL-12p40, following the indications of each manufacturer. Cells were cultured with concanavalin A (3·5 μg/well) for 24 hr for generation of IL-10 and IFN-γ or with SAC suspension (1 : 5000) for 48 hr for generation of TNF-α and IL-12. Data from the standard curve were submitted to linear regression analysis and the results of samples were expressed as pg/ml: (a) *in vitro* TNF-α production, (b) *in vitro* IFN-γ production, (c) *in vitro* IL-12p40 production and (d) *in vitro* IL-10 production. Results are expressed as means and standard errors. Statistical analysis (2 × 2 factorial) showed that AIRmax produced higher levels of TNF-α, IL-12 and IFN-γ than AIRmin and that it was not affected by gender in either strain.

resistant to the development of a variety of transplantable or chemically induced tumours than the counterpart AIRmin mice.[2,3,6] Considering that NK cells are able to kill some types of tumour cells and are associated with resistance against melanoma metastasis[28] the present work was developed to evaluate quantitative and qualitative differences between the NK cells of these two strains of mice. We also evaluated the *in vitro* production of some cytokines that are commonly involved with NK activity.

We first observed higher lytic activity against Yac.1 target cells in normal AIRmax mice than AIRmin mice. We also observed that AIRmax mice have slightly more $CD49b^+$ cells than AIRmin, and although this difference was statistically significant, it did not appear to be sufficient to explain the qualitative difference between these two strains.

In this work we have used a Pan-NK antibody (DX5, anti-CD49b) that reacts with an integrin α_2[29] because both AIRmax and AIRmin mice were developed by cross-breeding eight different parental inbred strains (A/J, DBA/2J, P/J, SWR/J, SJL/J, CBA/J, BALB/cJ and C57BL/6J)[1] and that antibody is able to react with cells of several inbred strains of mice. The relative number of $CD49b^+$ cells in our animals ranged from 1·4 to 2·5%, which was lower than the previously reported 5–10% in other strains.[18,30] However, these values appear to be a feature of AIR mice because the antibody used in this assay reacted at the same levels as observed by others when tested with spleen cells of BALB/c mice (data not shown), indicating that there were no problems with its reactivity. Arase *et al.*[29] observed that the antibody DX5 reacts with CD49b only when it is expressed in high density on the cell surface and that the progressive loss of this antigen is related to cell division. Thus, it is possible to speculate that the selective process for obtaining AIR mice has resulted in under-expression of CD49b compared to the parental lineages. Since NK activity depends on close contact between effector and target cells, we must consider that, besides the quantitative difference between the strains, NK cells of AIRmin mice could show impaired ability in cell adhesion.[32–34] However, the expression of cell adhesion molecules as well as antigen receptors has to be further analysed.

Although the MHC-unrestricted cytotoxic activity of NK cells does not depend on previous stimulation[15–18] these cells are strongly influenced by cytokines to increase the antitumour activity. Besides the IL-2, which stimulates the killer activity by generating lymphokine-activated killer cells,[31,35] IL-12 is one of the main NK-cell-stimulating cytokines.[36] In our study, we did not detect IL-12 dimeric protein p70 (data not shown), but we observed that AIRmax mice produced higher levels of IL-12p40 in response to SAC. This protein corresponds to the α-chain subunit (molecular weight 40 000) of a whole IL-12 and is usually produced during the early phase of immune response by both antigen-presenting cells and Th1 cells.[36–38] Although

its biological significance is not completely understood, some data indicate that it could antagonize IL-12 through competitive binding with IL-12 receptors.[38] On the other hand, Jun Há *et al.*[36] have observed that this protein is important for the chemoattraction of macrophages to the tumour site, playing a role in the organization of the early immune response, which could explain its higher levels in AIRmax animals.

Tumour necrosis factor-α, which is produced by a variety of cells such mastocytes, neutrophils, NK cells and mainly monocytes/macrophages, is also associated with the inflammatory process[22,39] and many authors have shown its participation in the antitumour immune response.[39–41] Besides the direct cytotoxic activity of TNF-α on some tumour cells[42] it can also intensify the antitumour immune response by activating NK cells or by inducing IL-12 production.[43] Higher levels of TNF-α in AIRmax are in accordance with their higher NK activity but they apparently do not affect the production of biologically active IL-12. Since we did not detect biologically active IL-12 in the culture supernatant and the subunit p40 is a common chain of IL-23 and IL-27, we speculate that greater NK activity can also be associated with production of these cytokines. Interleukin-23 is a cytokine of the IL-12 family and is able to stimulate the proliferation and activation of T lymphocytes, NK cells and macrophages, as well as cytokine production by these cells,[44] whereas IL-27 has recently been identified as sharing the IL-12p40 chain and inducing proliferation of naive T cells; it is a strong inducer of IFN-γ production.[45]

Natural killer activity and acute inflammatory responsiveness of AIRmax mice are also compatible with their capacity for *in vitro* production of IFN-γ. Data of TNF-α and IFN-γ production are also in agreement with those of Vigar *et al.*[14] who observed that AIRmax mice with pristane-induced arthritis produce more TNF-α than AIRmin mice, whereas Carneiro *et al.*[46] observed that AIRmax animals treated intraperitoneally with *Bothops jararaca* snake venom produce higher levels of TNF-α and IFN-γ than AIRmin mice.

In addition to the effects on NK activity, *in vivo* production of the above-mentioned cytokines can activate $CD8^+$ CTLs, which are able to kill both virus-infected cells[25] and tumour cells.[47] In contrast to NK cells, CTLs depend on the recognition of antigens associated with MHC class I molecules.[25] Levels of $CD3^+$ $CD8^+$ cells in the spleens of both AIRmax and AIRmin mice are in accordance with those observed in other normal inbred mice, but comparison of the number of only these two strains demonstrated that AIRmax have more circulating $CD8^+$ cells than AIRmin. Our results suggest that this difference can be the result of the ability of these animals to produce pro-inflammatory cytokines. This finding suggests that higher resistance of these animals to tumour development can be the result of the ensemble action of NK and CTL cells because these two populations of effector cells are able to destroy distinct target cells. It was to be considered that, in contrast to humans and rats, mouse NK cells do not express $CD8^+$ marker.[16]

Although the production of TNF-α, IFN-γ and IL-12p40 suggests the prevalence of Th1 lymphocytes in AIRmax animals, we did not observe alterations in the *in vitro* IL-10 production. IL-10 is an important regulatory cytokine produced by macrophages,[48,49] NK cells[18] and, mainly, Treg cells.[50] Their main actions are the inhibition of antigen-presenting cell activity,[48] MHC molecule expression,[49] costimulatory molecule expression,[51] and microbicidal activity of macrophages and neutrophils.[52] The presence of IL-10 however, can enhance NK activity by diminishing the class I MHC molecule expression on target cells. Class I MHC structures are the ligands for inhibitory Ly 49 and CD94/NKG2 receptors on NK cells.[53] Moreover, some works have shown that IL-10 can exert stimulatory effects on CTL, as demonstrated by Fuji *et al.*[54] These authors observed that administration of IL-10 to mice stimulates CTL proliferation and the development of memory tumour-specific $CD8^+$ cells, which can rapidly respond to antigens.

Taken together, our results suggest that resistance to development of neoplasias reported in AIRmax animals can be the result of polarization of other elements of the innate response (together with inflammatory ability), by selecting the genes related to the control of NK activity and production of pro-inflammatory cytokines involved in the activation of these cells.

AIRmax and AIRmin mice present extreme and opposite phenotypes but are genetically heterogeneous, thus offering a unique model for the identification of common genetic modifiers of inflammation and cancer.[4] In this way, more studies are being performed to verify whether the difference in NK activity is the result of the differential expression of activating or inhibiting receptors or if the AIRmin NK cells present a defective response to the activation stimuli.

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