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Defects in the regulation of anti-DNA antibody production in aged lupus-prone (NZB × NZW)F_1 mice: analysis of T-cell lymphokine synthesis

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SUMMARY

(NZB × NZW)F_1 (B/W) mice spontaneously develop a lupus-like syndrome characterized by an increased level of autoantibodies in old mice. We analysed the role of T cells in the regulation of anti-DNA antibody production by B cells *in vitro* as a function of age. In cultures of old mouse T and B cells, IgG and IgM anti-DNA antibodies were synthesized at high levels, in contrast to consistantly lower amounts, particularly of IgG, measured in cultures of young mouse cells. Addition of young mouse T cells to old B cells inhibited IgG, but not IgM, anti-DNA production, whereas T cells from old mice stimulated IgG synthesis by young mouse B cells. Addition of supernatants harvested from concanavalin A (Con A)-stimulated T cells to B-cell cultures induced similar effects. Therefore, we evaluated possible modifications of lymphokine synthesis compared to that of the healthy NZW parent. T cells from old mice were able to secrete normal levels of interferon-γ (IFN-γ) and interleukin (IL)-10; however, secretion of IL-2 and IL-4 was dramatically decreased. Semi-quantitative polymerase chain reaction analysis of constitutive RNA messengers showed increased IFN-γ levels in young and old B/W mice, and normal IL-10 mRNA levels in young and higher levels in old mice. Constitutive IL-2 and IL-4 mRNA were detected only after Con A stimulation and their levels decreased in old compared to young B/W mice; in particular IL-2 mRNA was considerably lower in old B/W than in control NZW mice. Taken together, these results suggest that, despite constitutive T-cell abnormalities, young B/W mice are able partially to control their lymphokine production, whereas aged mice exhibit a deficient synthesis, associated with an increased capacity to produce IFN-γ.

INTRODUCTION

The (NZB × NZW)F_1 (B/W) mouse, a murine model of systemic lupus erythematosus, is characterized by the spontaneous lack of tolerance to several self-antigens. B-cell hyperactivity with the subsequent appearance of antibodies to nuclear antigens leads to fatal, immune complex-mediated glomerulonephritis.[1] Although a polyclonal B-cell activation is observed in the early stages of the disease,[2] it has been established that T cells play a crucial role in the pathogenesis of lupus, as confirmed by the active role of $CD4^+$ T cells in the increased production of IgG antibody[3] or by suppression of the disease in anti-CD4-treated B/W mice.[4,5]

Imbalanced or defective lymphokine production could lead to major perturbations of the immune system. Indeed, a marked deficiency in *in vitro* interleukin (IL)-2 production has been described in lupus-prone mice.[6,7] Furthermore, studies performed on patients suffering from lupus have suggested that defects in IL-2 secretion[8] and low levels of high-affinity IL-2 receptors[9] might contribute to the development of the disease by decreasing suppressor-cell activation and function. Moreover, in *lpr* mice an abundant abnormal T-cell subset expressed several cytokines associated with inflammatory responses, including interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and TNF-β and IL-6.[10]

In the present study on B/W mice, we investigated the influence of T cells on the *in vitro* modulation of autoantibody synthesis, as well as the profile of their lymphokine secretion and gene expression.

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Abbreviations: B/W, (NZB × NZW)F_1; Con A, concanavalin A; ELISA, enzyme-linked immunosorbent assay; HPRT, hypoxanthine phosphoribosyl-transferase; [^{3}H]TdR, tritiated thymidine; IFN-γ, interferon-γ; IL, interleukin; LPS, lipopolysaccharide; mAb, monoclonal antibody; PCR, polymerase chain reaction; SN, supernatant.

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MATERIALS AND METHODS

Mice

Female NZB mice were obtained from the Institut Pasteur's (Paris, France) animal colony, and male NZW mice from the Centre de Sélection et d'Elevage des Animaux de Laboratoire (CNRS, Orléans, France). B/W mice were bred in our animal facilities. Young female mice (2–3 months old) did not show

any signs of disease and old female mice (8–9 months old) exhibited proteinuria and high levels of anti-DNA antibodies.[11] Male NZW mice (6–9 months old) have been described as phenotypically normal with a mean survival very similar to those of normal mice.[1]

Antibodies

The following monoclonal antibodies (mAb) were used throughout the study: anti-Thy-1.2 (J1J48), anti-CD4 (172-4) and anti-CD8 (3.1.55). For cytokine-specific enzyme-linked immunosorbent assay (ELISA), the unlabelled rat mAb directed against murine cytokines used were: anti-IFN-γ (R46A2; a kind gift from Dr Claude Leclerc, Institut Pasteur), anti-IL-2 (JES-61A12), anti-IL-4 (BVD4-1D11) and anti-IL-10 (JES-52A5); and the biotinylated rat mAb were: anti-IFN-γ (AN-18.17.24), anti-IL-2 (JES6-5H4), anti-IL-4 (BVD6-2462) and anti-IL-10 (SXC1). All mAb clones were obtained from DNAX Research Institute for Molecular and Cellular Biology (Palo Alto, CA) and were prepared in the Unité d'Immunoparasitologie. The specificities of these mAb have been described elsewhere.[12,13]

Cell suspensions and cultures

Cell suspensions were prepared from spleens and red blood cells were removed by hypotonic shock. All cultures were carried out in a complete medium [RPMI-1640 medium containing 5×10^{-5} M 2-mercaptoethanol (2-ME), 4 mM glutamine, 1 mM pyruvate, 5 IU/ml penicillin, 50 μg/ml streptomycin, and 10% fetal calf serum (FCS) (Gibco BRL, Grand Island, NY)]. In some experiments adherent cells were removed by incubating spleen cells on Petri dishes. Purified splenic T cells were obtained by two cycles of negative panning using goat anti-mouse immunoglobulin, and were >97% pure, as scored by fluorescence staining in a FACScan flow cytometer (Becton Dickinson, San José, CA). B-cell-enriched populations were obtained by cytotoxic treatment using mAb anti-Thy-1.2 or a mixture of mAb anti-CD4 and anti-CD8, and guinea-pig serum as a source of complement; they contained less than 5% T cells, as scored by fluorescence-activated cell sorter (FACS).

The *in vitro* synthesis of anti-DNA antibody was evaluated in supernatant (SN) of co-cultures of 10^6 B cells with 10^6 T cells in 1 ml of complete medium, during 6 days at 37°. In some experiments, 10^6 B cells were cultured with 25% SN derived from 10^6 T cells stimulated by Con A (5 μg/ml) for 24 hr.

For the cell proliferation assay, 10^6 cells/ml were distributed in flat-bottomed 96-well plates and incubated for 72 hr in the absence or presence of mitogens [5 μg/ml Con A or 50 μg/ml lipopolysaccharide (LPS)]. [^{3}H]thymidine ([^{3}H]TdR); 0·5 μCi/well; Amersham Int., Buckinghamshire, UK) was added 16 hr prior to harvesting and the amount of radioactivity incorporated was determined in a β-counter.

Detection of anti-DNA antibodies

ELISA were performed to evaluate antibodies directed against DNA, as described previously in detail,[14] using β-galactosidase-conjugated goat anti-mouse IgM, IgG1, IgG2a, IgG2b and IgG3 (Southern Biotechnology Associates Inc., Birmingham, AL). Enzyme activity was developed with o-nitrophenyl-β-D-galactopyranoside (ONPG; Sigma, St Louis, MO). The optical density (OD) was measured at 414 nm in an automatic reader (Dynatech, Marnes-la-Coquette, France). In order to detect the low concentrations of anti-DNA antibodies in SN, the fluorogenic substrate, 4-methylumbelliferyl-β-D-galactopyranoside (MUG; Sigma)[15] was used instead of ONPG, and arbitrary fluorescence units were estimated using a Titertech Fluoroskan (Labsystems, Life Science Int., Cergy-Pontoise, France).

Cytokine-specific ELISA

SN obtained from 4×10^6 T cells incubated with 5 μg/ml Con A were harvested after 24, 48 and 72 hr of culture, centrifuged and stored at −20° until tested. To measure the presence of cytokines in the SN, a sandwich ELISA using anti-cytokine mAb, either unlabelled for capture or biotinylated for detection, was performed. Avidin-peroxidase (Sigma) was added and reactions were revealed with o-phenylenediamine (Sigma). OD were read at 490 nm and 650 nm by an automatic reader. Values were calculated by reference to standard curves constructed using known concentrations of murine recombinant (r)IFN-γ (Pharmingen, San Diego, CA) or lymphokines derived from supernatants of the P815 mastocytoma cell line transfected with the IL-2 gene, LT1-4 lymphoma transfected with the IL-4 gene or J558 B-cell hybridoma transfected with the IL-10 gene.[16]

RNA isolation and reverse transcription

Total cellular RNA from 10^6 splenic T cells, either freshly isolated or Con A-stimulated (6 hr), was obtained using guanidium thiocyanate and phenol–chloroform extractions. Total RNA from the anti-conalbumin T-helper cell type-2 (Th2) clone, D10.G4.1 (D10; I-A^k), or the anti-keyhole limpet haemocyanin Th1 clone, HDK1 (I-A^d), stimulated with the respective antigen for 6 hr, was used to construct standard curves for interleukin transcripts.[17] cDNA was synthesized from RNA samples using Moloney murine leukaemia virus reverse transcriptase (Gibco) in the presence of oligo (dT) (Pharmacia Fine Chemicals, Uppsala, Sweden), as described elsewhere.[18]

Semi-quantitative polymerase chain reaction (PCR)

Analysis of cytokine gene expression and specificities of the primers and probes used have been described in detail elsewhere.[16,19] Briefly, 5 μl of cDNA was amplified using a mixture of sense and anti-sense primers (2 μM each), 2·5 μl of 10 × PCR buffer (100 mM Tris–HCl, pH 8·3, 500 mM KCl, 15 mM $MgCl_2$, 0·05% Tween-20), 0·5 μl of dNTP mixture (dGTP, dATP, dCTP and dTTP) (10 mM each), 14·4 μl of H_2O and 0·1 μl of Taq DNA polymerase (Pharmacia). PCR amplifications were done in a thermal cycler (GeneAmp 9600 PCR System, Perkin Elmer Cetus, St Quentin Yvelines, France) by one 2-min cycle at 92°, followed by 30 cycles, each of 10 seconds at 91°, 25 seconds at 59° and 25 seconds at 72°. PCR products from the samples being tested were then compared for their content of the house-keeping enzyme, hypoxanthine phosphoribosyltransferase (HPRT), in parallel with the products obtained from the amplification of known amounts of input RNA from D10, or HDK1 standard clones, followed by dot-blot hybridization with a specific HPRT internal probe labelled with [^{32}P-γ]ATP. Autoradiograms were analysed in a MASTERscan (BIONIS-CSPI, Richebourg, France). All experimental samples were corrected to the same HPRT–mRNA signal obtained on the exponential part of the standard

curve. Samples were then dot blotted and hybridized to confirm the HPRT level adjustment. After adjustment, cDNA samples were amplified for IFN-γ, IL-2, IL-4 or IL-10 mRNA using specific pairs of primers. PCR products were dot-blotted and hybridized with lymphokine-specific [^{32}P-γ]ATP-labelled internal probes. mRNA pg-equivalents were calculated for each sample after quantification of these final dot-blots from the linear part of the standard curves.

Statistical analyses

Student's *t*-test was applied to determine the statistical significance of differences observed.

RESULTS

In vitro regulation of anti-DNA antibody production by T cells from B/W mice

In old B/W mouse sera, IgG anti-DNA antibody levels sharply increased, with a preference for the IgG2a and IgG2b isotypes (Fig. 1). To examine the T-cell involvement in the production and regulation of anti-DNA autoantibodies, we first analysed the spontaneous proliferative response, in the absence of exogenous stimuli, of cultures and co-cultures of isolated T- and B-cell populations derived from young and old B/W mice.

As can be seen in Fig. 2, the spontaneous uptake of [^{3}H]TdR by T and B cells was significantly higher in cells from old than from young mice. However, after mitogen stimulation, while B cells from both young and old mice responded similary to LPS (Δ c.p.m. = 40 754 ± 16 132 and 44 299 ± 15 609, respectively), T-cell response to Con A was significantly less for old (Δ c.p.m. = 14 542 ± 5749) compared to young mouse T cells (Δ c.p.m. = 49 195 ± 24 818). In B–T-cell co-cultures the spontaneous proliferation was significantly lower with young mouse B cells and autologous T cells than in young mouse B and old mouse T cells (Fig. 2; YB + YT versus YB + OT). In contrast, proliferation in co-cultures of old mouse B cells with T cells from either old or young mice, did not differ significantly (Fig. 2; OB + OT versus OB + YT).

IgM and IgG anti-DNA antibody synthesis in these co-culture SN were measured by ELISA. Figure 3 shows that young mouse B cells cultured with autologous T cells secreted IgG anti-DNA antibodies with an activity considerably lower

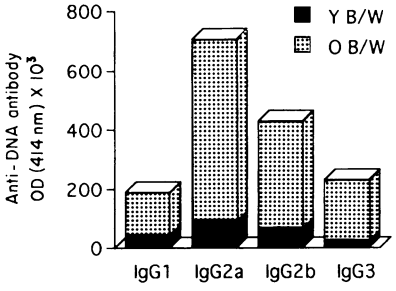

Figure 1. IgG isotypes of anti-DNA antibodies in sera of young (Y) (n = 10) and old (○) (n = 10) B/W mice. Individual sera were tested at 1 : 100 dilution on a DNA-coated plate and revealed with β-galactosidase-labelled antibodies specific for mouse IgG isotypes. Values are the mean OD measured in each group of mice.

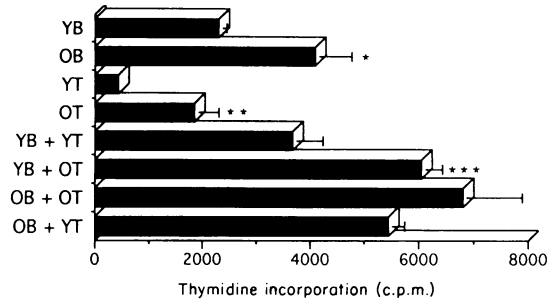

Figure 2. Spontaneous proliferation of B/W cells. Single-cell suspensions of purified B and T cells were prepared from spleen of young (YB, YT) and old (OB, OT) B/W mice. B or T cells alone, or mixed young, or old B/W mouse B- and T-cell populations (10^5 cells/well) were cultured for 3 days in the absence of mitogen. Results are expressed as the means ± SEM of six separate experiments. $*P \leqslant 0{\cdot}02$, significantly different from YB cell culture; $**P \leqslant 0{\cdot}02$ significantly different from YT cell culture; $***P \leqslant 0{\cdot}001$ significantly differently from YB + YT co-cultures, as determined by Student's *t*-test.

(nine times the fluorescence arbitrary units) than that measured in co-cultures with old mouse T cells (Fig. 3a). However, IgM anti-DNA antibody secretion was similar in both co-cultures (Fig. 3a). This increase seemed specific for IgG anti-DNA antibodies, since the total secreted IgG was only doubled (data not shown). Similarly, co-cultures of old mouse B cells with young mouse T cells secreted IgG anti-DNA antibodies with a lower activity than that measured in autologous old mouse cell

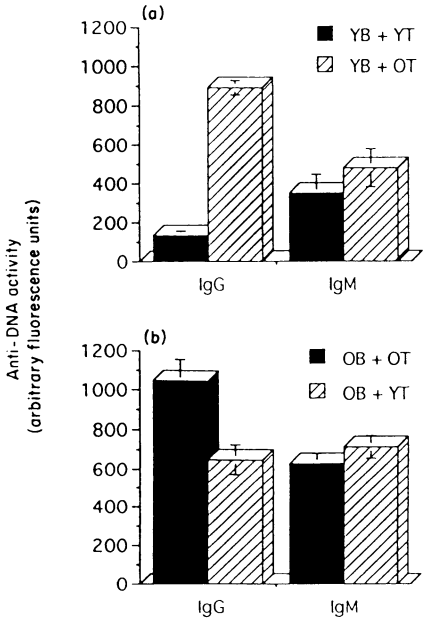

Figure 3. Autoantibody synthesis in co-cultures of B and T cells from B/W mice. (a) Pools of B cells (10^6/well) from young mice (n = 2) were co-cultured with pools of autologous T cells (10^6/well) or with those from old mice (n = 3), for 6 days. (b) Pools of B cells from old mice (n = 3) were co-cultured with pools of autologous T cells or with those from young mice (n = 2) for 6 days. The supernatants were tested by ELISA for IgG and IgM anti-DNA antibodies. The values shown are means ± SEM of four separate experiments.

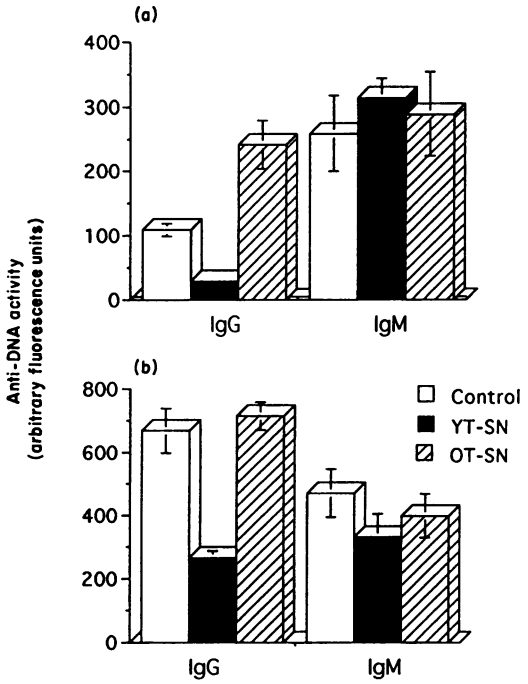

Figure 4. Effect of soluble factor(s) from activated T cells on the immunoglobulin B-cell synthesis. Pools of B cells (10^6/well) from young (a) (n = 2) or old (b) (n = 3) B/W mice were cultured for 6 days in the presence of SN from Con A-stimulated T cells from young (YT-SN) or old (OT-SN) mice. A control B-cell culture was performed in the absence of SN. The supernatants were tested by ELISA for IgG and IgM anti-DNA antibodies. Means ± SEM of four separate experiments.

cultures (Fig. 3b), which was in the same range as that in co-cultures of young mouse B cells with old mouse T cells (Fig. 3a). IgM anti-DNA activities were similar in both co-cultures (Fig. 3b).

Effect of soluble mediators from activated T cells on anti-DNA antibody production

Since IgG anti-DNA antibody synthesis was modulated by the presence of T cells, we investigated the possible role of T-cell-soluble mediators. Compared to the spontaneous secretion by young or old mouse B cells (control), addition of Con A–SN from young T cells (YT-SN) to B cells from either young (Fig. 4a) and old mice (Fig. 4b) decreased the IgG anti-DNA antibody activity by 70% and 60%, respectively. In contrast, addition of Con A–SN obtained from old mouse T cells (OT-SN) increased the IgG anti-DNA antibody secretion by young mouse B cells (Fig 4a) but did not modify that of old mouse B cells (Fig. 4b). Addition of Con A–SN from both young and old mice slightly increased the IgM anti-DNA antibody secretion by young mouse B cells (Fig. 4a) and slightly decreased this secretion by old mouse cells (Fig. 4b).

Lymphokine secretion by T cells

IFN-γ, IL-10, IL-2 and IL-4 T-cell secretions were titrated by ELISA in the Con A–SN from young and old B/W, and from old NZW for comparison, after 24, 48 and 72 hr of culture. As shown on Fig. 5a, IFN-γ secretions were similar in the three groups, with similar levels at 24 and 48 hr that decreased at 72 hr. In contrast, IL-10 release increased with time; after 48 hr

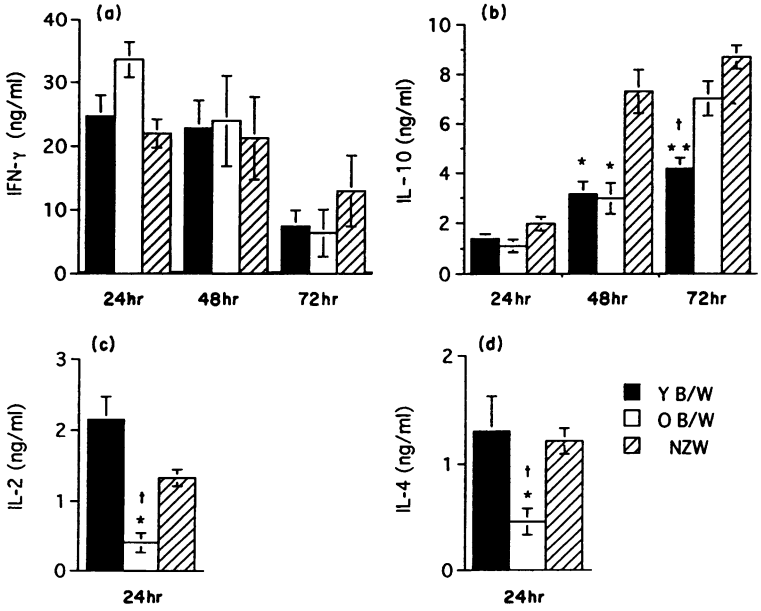

Figure 5. Lymphokine production by T cells from B/W mice. Single T-cell suspensions were prepared from young (■) and old B/W (□) and NZW (▨). They were cultured with Con A for 24, 48 and 72 hr. SN were tested by ELISA at each time-point of culture for the presence of IFN-γ (a) and IL-10 (b) and after 24 hr for the presence of IL-2 (c) and IL-4 (d). Mean values ± SEM of four or five individual mouse SN in each group are shown. (b) $*P \leqslant 0{\cdot}007$, $**P \leqslant 0{\cdot}01$, significantly different from NZW T-cell IL-10 secretion, $\dagger P \leqslant 0{\cdot}025$, significantly different from old B/W T-cell IL-10 secretion; (c) $*P \leqslant 0{\cdot}002$, $\dagger P \leqslant 0{\cdot}036$, significantly different from, respectively, young B/W and NZW T-cell IL-2 secretion; (d) $*P \leqslant 0{\cdot}047$, $\dagger P \leqslant 0{\cdot}0046$, significantly different from, respectively, young B/W and NZW T-cell IL-4 secretion.

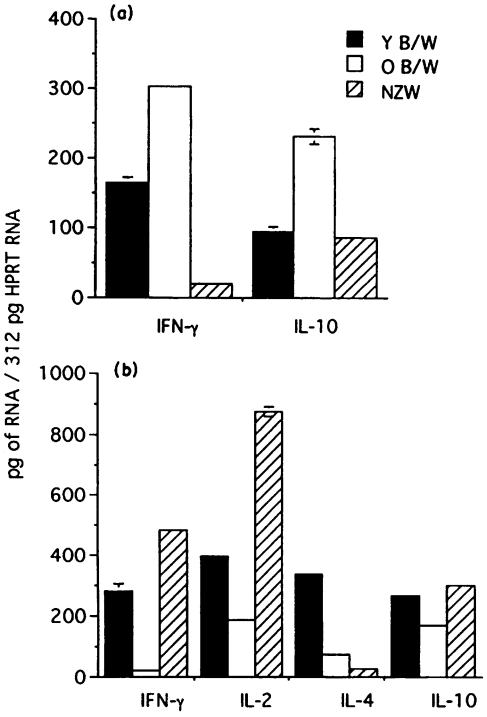

Figure 6. Semi-quantitative analysis of IFN-γ, IL-2, IL-4 and IL-10 gene expression in T cells. RNA was extracted from pools of 10^6 freshly purified (a) and Con A-stimulated (b) T cells from young (■) ($n = 2$) or old B/W (□) ($n = 3$) or NZW (▨) ($n = 2$) mice. Different RNA dilutions were co-reverse transcribed in parallel with known concentrations of RNA obtained from a standard Th1-cell line (HDK1). All samples were amplified by PCR using specific primers for the housekeeping HPRT gene, followed by a duplicate dot-blot hybridization with an HPRT internal probe labelled with [^{32}P-γ]ATP. All samples, adjusted to equivalent amounts of 312 pg of HPRT transcript (see the Materials and Methods) were amplified with IFN-γ-, IL-2-, IL-4-, IL-10-specific primers, in parallel with cDNA from standard TH1- or TH2-cell lines. PCR products were dot-blotted in duplicate and hybridized with lymphokine specific labelled internal probes. Autoradiographs were analysed in a MASTERscan. Results are expressed as RNA pg equivalents for each lymphokine calculated from the respective Th1 and Th2 standard curves. Errors bars represent variations between two samples of the same experiment for each lymphokine analysed.

of stimulation, the level secreted by young and old B/W T cells was significantly lower than that of NZW (Fig. 5b); and after 72 hr, secretion by old B/W had increased to the NZW level, whereas that of young B/W remained lower (Fig. 5b). After 24 hr, IL-2 and IL-4 secretions by young B/W and NZW T cells were similar (Fig. 5c, d), while those of old B/W were significantly lower.

Quantification of constitutive lymphokine mRNA expression in B/W T cells

cDNA preparations from freshly purified T cells were initially titrated and standardized to 312 pg-equivalents of the house keeping (HPRT) gene, to correct for differential mRNA expression between the samples. Thus, levels of IL-2, IFN-γ, IL-4 and IL-10 gene expression were fitted to a standard curve of known amounts of mRNA from D10 (Th2) or HDK1 (Th1) cell lines. The amounts of constitutive IFN-γ mRNA increased with ageing from 165 ± 7·4 to 303 ± 1·72 mRNA pg-equivalents, and those of IL-10 from 94·3 ± 7·3 to 231·5 ± 2·7 mRNA pg-equivalents (Fig. 6a). Compared to old NZW, the mRNA expression in old B/W mice was 15 times higher for IFN-γ and three times for IL-10 (Fig. 6a). IL-2 and IL-4 mRNA were not detectable in any T-cell preparation analysed (data not shown).

Cytokine expression in T cells following in vitro Con A stimulation

The patterns of IFN-γ, IL-2, IL-4 and IL-10 gene expression were examined in T cells stimulated with Con A for 6 hr. mRNA expression was decreased in old compared to young B/W mice for all lymphokines, particularly IFN-γ, IL-2 and IL-4 (Fig. 6b). Compared to old NZW mice, the IFN-γ, IL-2 and IL-10 mRNA levels in old B/W mice were decreased, respectively, by 96%, 78% and 43%, demonstrating the dramatic effect of Con A on IFN-γ transcription. In contrast, IL-4 mRNA levels, which were considerably higher in young mice, decreased in old B/W to almost the same level as that of NZW (Fig. 6b).

DISCUSSION

In the present study, we observed that young B/W mouse T cells were not able to induce IgG anti-DNA production by either autologous or old mouse B cells. In contrast, T cells from old mice stimulated a high level antibody production by both young and old mouse B cells. The IgM anti-DNA antibody synthesis was not influenced by T cells from either young or old mice, suggesting a spontaneous polyclonal activation due to an intrinsic B-cell defect.[2,3] Thus, class-specific regulatory T cells[20] may function as an important regulatory mechanism to avoid the expansion of autoreactive IgG-secreting B-cell clones in young mice. However, in old mice, T cells seemed to present abnormalities, providing excessive help to young mouse B cells and isotype switching to IgG. These effects were more effective in B cells cultured with T cells than with T-cell-derived lymphokines, especially with young B cells. This is probably due to the degree of B-cell activation, since it has been demonstrated that resting B cells require contact with T-helper cells in order to respond to lymphokines by proliferation and differentiation.[21]

Despite their active regulatory mechanism on B cells, young mouse T cells showed an abnormally high level of constitutive IFN-γ mRNA, and this level increased with ageing. This lymphokine can mediate isotype switching to IgG2a,[22] the dominant anti-DNA antibody isotype observed in old B/W sera and kidney eluates.[23] In addition, IFN-γ treatment in B/W mice accelerated the development of the fatal glomerulonephritis, which could subsequently be reversed by the administration of anti-IFN-γ antibodies.[24]

Although in young mice constitutive IL-10 mRNA levels were normal, in old mice it increased, although by the levels less than IFN-γ did. IL-10 secretion, however, was significantly lower in young and delayed in old mice compared to that of controls. Since IL-10 is known to inhibit IFN-γ synthesis at the transcriptional and post-transcriptional levels,[25,26] these results suggest that, despite an increased amount of IL-10 mRNA, its secretion was probably not sufficient to down-regulate IFN-γ expression in old mice. On the other hand, since IL-10 may act

by inhibiting antigen presentation,[27,28] the early defect in IL-10 secretion could result in an abnormal regulation that continuously favours the stimulation of autoreactive IFN-γ-producing cells. However, since IL-10 administration accelerates clinical symptons and anti-IL-10 treatment is beneficial for B/W mice,[29] other mechanisms must be implicated in their immune dysregulation.

Paradoxically, under *in vitro* Con A stimulation, we observed an abrupt down-regulation of IFN-γ, whereas the IL-10 transcript level was unchanged in old B/W T cells. This effect, which occurred only in old B/W cells, seems to be a temporary *in vitro* phenemonon, since the IFN-γ secretion induced by Con A was normal. Such a decrease in constitutive IFN-γ level was also observed after phorbol myristate acetate (PMA) stimulation of the $CD4^-/CD8^-$ T-cell subset of *lpr* mice, which present an unusual coexpression of Thy-1 and B220 molecules.[30]

IL-2 mRNA expression, as well as IL-2 secretion, by old B/W T cells was much lower than that of controls, probably due to the impaired ability to proliferate in response to Con A stimulation. These defects have been reported in cells from old mice carrying the *lpr* gene.[6,7] Nevertheless, *in vitro* IL-2 production has been obtained by stimulating B/W and MRL-*lpr/lpr* mouse lymphocytes with other chemicals,[31,32] suggesting that IL-2-gene expression may be differentially induced by various culture conditions. Indeed, the IL-2 decreased signal, which reflects an *in vitro* post-activation refractoriness of T cells from old B/W mice, was not observed with T cells from young mice, which produced a normal level of this lymphokine. Similarly, the IL-4 defect level in mRNA expression and secretion was detected only in old B/W T cells.

In conclusion, despite the early presence of abnormalities at the 'preautoimmune' repertoire state, these T cells displayed an active down-regulatory effect on autoreactive IgG-secreting B cells, in young B/W mice. This regulatory capacity was lost with ageing. The abundant expression of constitutive IFN-γ on one hand, and the lower IL-4 secretion on the other, suggest a break in the Th1/Th2 subset balance of old B/W cells. Despite the defective IL-2 expression, probably due to differences in the regulation of IL-2 and IFN-γ genes,[33] this imbalance seems to favour the IFN-γ-producing cells, a hypothesis sustained by the predominance of IgG2a antibody synthesis.

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