Immunology 1998 **95** 377–382

Involvement of *c-myc* in the resistance of non-obese diabetic mice to glucocorticoid-induced apoptosis

T. C. MARTINS* & A. P. ÁGUAS*† *Institute for Molecular and Cellular Biology, and †Department of Anatomy of the Abel Salazar Institute for the Biomedical Sciences of the University of Porto, Porto, Portugal

SUMMARY

Non-obese diabetic (NOD) mice spontaneously develop insulin-dependent diabetes mellitus (IDDM) as a consequence of autoimmune aggression of β cells of the endocrine pancreas by T cells. T lymphocytes of NOD mice are resistant to apoptosis induced by glucocorticoids, or by starving or DNA-damaging treatments, a feature that was interpreted as being linked to escape of autoreactive T cells from thymic negative selection. *c-myc* is one of the gene targets of glucocorticoids (GC), its expression being down-regulated by the activated GC–GC receptor complex. We investigated here whether expression of Myc protein, in response to dexamethasone stimulation, was the same in NOD mice and in non-autoimmune strains, namely NON, BALB/c and C57Bl.6. We found a consistent increase in the levels of Myc protein after GC-treatment of lymphocytes of NOD mice, a finding that was in contrast to the down-regulation of *c-myc* that we observed in lymphocytes from mice not prone to diabetes. We also report that, rather than a absolute resistance to GC-induced cell death, NOD mice display a delayed apoptotic response to GC. We propose that the resistance of NOD mice lymphocytes to GC-induced apoptosis is because of inhibition of the repressive action of GC-GR complexes at the level of *c-myc* transcription. This deficient action of GC–GR results in increased production of nuclear Myc protein, peculiar to NOD mice cells, following their treatment with GC.

INTRODUCTION

Apoptosis is an important process in the maintenance of the homeostasis of the immune system, having a determinant role in the negative selection of lymphocytes, as well as in deletion of activated lymphoid cells after an immune response.[1–3] Several studies showed that glucocorticoids (GC) modulate thymic selection of T lymphocytes, high levels of thymic endogenous GC being responsible for deletion of T cells by neglect.[2–4] In fact, one of the earliest observed effects of GC treatment in rodents is thymic involution.

Non-obese diabetic (NOD) mice spontaneously develop insulin-dependent diabetes mellitus (IDDM) as a consequence of autoimmune aggression of β cells of the endocrine pancreas by T cells.[5–9] T lymphocytes of NOD mice are resistant to apoptosis induced by glucocorticoids, or by starving or DNA-damaging treatments, a feature that was interpreted as being linked to escape of autoreactive T cells from thymic negative selection.[10–13] The mechanism of this resistance has not been defined as yet. It has been proposed that it is because of an increased expression of the *bcl-2* gene;[12] however, there is evidence that the molecule responsible for this resistance acts up-stream of the *bcl-2* gene and is common to three distinct apoptotic pathways: glucocorticoid-, starving-, and DNA damage-induced cell death.[9] Interaction between GC and GC receptors (GR) is required for apoptosis to occur. After entering the cells, GC binds to GR, and the receptor is thus 'activated', and translocates to the nucleus where it exerts a repressive action over essential gene products.[14–16] One of the targets of the GC-GR complex is c-*myc,* its expression being down-regulated. This down-regulation of the *myc* gene and the subsequent lowering of the levels of Myc protein are essential for the induction of apoptosis by GC.[16,17] Interestingly, Myc is also involved in DNA damage-induced apoptosis, interacting with p53, and it is also involved in serum-deprivation-induced death of lymphocytes,[18] which led us to suspect its involvement in apoptosis resistance of NOD mouse lymphocytes.

We investigated here whether expression of Myc protein, in response to dexamethasone stimulation, was the same in NOD mice and in non-autoimmune strains, namely NON, BALB/c and C57Bl.6. We found a consistent increase in the levels of Myc protein after GC-treatment of lymphocytes of NOD mice, a finding that was in contrast with the down-

Received 31 March 1998; revised 29 June 1998; accepted 29 June 1998.

Abbreviations: Dex, dexamethasone; GC, glucocorticoids; GR, glucocorticoid receptor; NOD, non-obese diabetic; tunel, TdT-mediated dUTP-biotin nick end-labelling.

Correspondence: Dr T. C. Martins, Institute for Molecular and Cellular Biology, University of Porto, Rua do Campo Alegre 823, 4150 Porto, Portugal.

regulation of *c-myc* that we observed in lymphocytes from mice not prone to diabetes. This investigation thus reveals a mechanism underlying the resistance of NOD mouse lymphocytes to GC-induced apoptosis: the phenomenon is because of inhibition of the repressive action of GC–GR complexes at the level of *c-myc* transcription. This deficient action of GC–GR results in increased production of nuclear Myc protein, peculiar to NOD mouse cells, following their treatment with GC.

MATERIALS AND METHODS

Mice

Breeding nuclei of NOD/Lt and NON/Lt mice were established in this research centre from animals purchased from the Jackson Laboratory (Bar Harbour, PA). NOD mice spontaneously develop type 1 diabetes, whereas NON mice are a related diabetes-resistant strain. Non-autoimmune BALB/c and C57Bl.6 breeding pairs were supplied by a local breeder, the Gulbenkian Institute of Science (Oeiras, Portugal). Mice were kept in the animal facilities of our research institute under standard housing conditions, and were fed commercial chow and acidified water.

Cell suspensions

A standard procedure was used to prepare exhaustive cell suspensions from spleen and thymus.[19] Viable cells were counted by means of a trypan blue exclusion test.

In vitro *dexamethasone (Dex) treatment of cells*

2×10^6 thymic or splenic cells from each of the murine strains were *in vitro* stimulated with 10^{-7} M dexamethasone (Dex) (Sigma Chemical Co., St Louis, MO) in RPMI-1640 medium (Gibco BRL Life Technologies Ltd, Paisley, UK) containing 10% fetal calf serum (FCS), 200 mM L-glutamine, 10 mM HEPES (all from Gibco BRL Life Technologies Ltd) and 50 μM 2-mercaptoethanol (2-ME; Sigma Chemical Co.) (37°, 5% CO_2). Controls consisted of untreated cells cultured in the same medium.

Assessment of glucocorticoid-induced thymic involution

NOD and C57Bl.6 mice were intraperitoneally injected with 0·2 mg Dex. 48 hr after injection thymic involution was assessed by viable cell counting (trypan blue exclusion).

Flow cytometric analysis of cell cycle by PI staining

2×10^6 thymic or splenic cells, either stimulated with Dex or untreated, were fixed and permeabilized in ethanol 70% (5 min, 4°) and washed in phosphate-buffered saline (PBS) before incubation in a propidium iodide (PI) (Sigma Chemical Co.) solution (50 μg/ml) in PBS, for 30 min (37°, 5% CO_2). The cell cycle was then analysed using a Becton Dickinson FACSort flow cytometer (Becton Dickinson, Mountain View, CA) interfaced to a Hewlett-Packard computer (Hewlett-Packard, CA). This method to identify apoptotic cells is modified from the one described by Telford and co-workers.[20]

Flow cytometric analysis of apoptosis by tunel

2×10^6 thymic or splenic cells, either stimulated with Dex or untreated, were first stained with the following monoclonal antibodies for cell surface markers: cychrome-labelled anti-CD4 and, PE-labelled anti-CD8, or PE-labelled anti-CD3 (all purchased from Pharmingen, San Diego, CA). Next, tunel assay was performed as described elsewhere.[21] Briefly, after fixation with paraformaldehyde, the cells were permeabilized with saponin and incubated with fluorescein isothyocyanate (FITC)-labelled TdT-dUTP (Boheringer Mannheim, cat. no. 1684795, Mannheim, Germany) for 30 min (5% CO_2, 37°). The staining pattern of the cells was analysed using a Becton Dickinson FACSort flow cytometer interfaced to a Hewlett-Packard computer.

Immunoblotting

2×10^6 thymic or splenic cells, either stimulated with Dex or untreated, were washed in PBS, collected by centrifugation and lysed and denatured for 10 min in Laemelli's sample buffer. Samples were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) on 10% polyacrylamide gels. Protein were then transferred to a nitrocellulose membrane (Schleicher & Schuell, Germany) using a semi-dry transfer apparatus (Bio-Rad, Hercules, CA). Membranes were incubated for 3 hr in 5% non-fat dried milk in PBS, followed by overnight incubation at 4° in primary antibody, diluted in 1% non-fat dried milk. Membranes were washed three times in PBS–0·2% Tween 20 and were incubated in donkey–anti-mouse horseradish peroxidase-conjugated secondary antibody (Amersham International, Amersham, UK) diluted in 1% non-fat dried milk. Membranes were washed three times in PBS–0·2% Tween 20 and developed by enhanced chemiluminescence system (Amersham) and exposed to CP-BU film (Agfa, Germany). Two different anti-myc antibodies were used: one purchased from Calbiochem (OP30; La Jolla, CA) and the other from Santa Cruz Biotechnologies (SC-42; Santa Cruz, CA). Both antibodies yielded similar results. Protein expression was also analysed by staining of gels with Coomassie Blue.

RESULTS

In vitro GC response of thymocytes and splenic cells

Thymocytes obtained from diabetes-prone NOD mice and non-autoimmune NON, BALB/c and C57Bl.6 mice were stimulated *in vitro* with Dex. Four hours after stimulation there was a consistent increase in the percentage of apoptotic thymocytes from NON and C57Bl.6 mice in comparison with cells from NOD mice; thymocytes from BALB/c mice showed a slight enhancement in apoptotic cells in response to GC. The apoptotic levels observed in thymocytes of NOD mice after Dex treatment were similar to the levels observed in untreated controls of the non-autoimmune strains (Fig. 1a). The differences in GC response between lymphocytes from NOD mice and from non-autoimmune strains were also observed when splenic cells were used. Six hours after Dex stimulation there was a pronounced increase in the fraction of splenic cells undergoing apoptosis in all three non-autoimmune strains (that is NON, BALB/c and C57Bl.6); the percentage of NOD lymphocytes undergoing apoptosis was similar to the control levels observed in the non-autoimmune strains (Fig. 1b). Thymocytes of autoimmunity-prone and normal mice were also stimulated with Dex *in vitro* for a longer period of time (12 hr and 24 hr). In these experiments, the percentage of apoptotic cells of NOD mice were similar to those observed

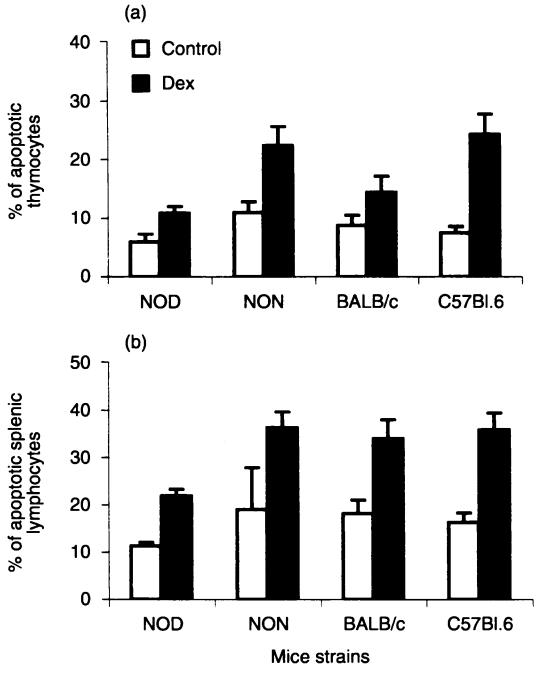

Figure 1. Comparison of the short-term glucocorticoid response of thymocytes and splenic cells of NOD mice and non-autoimmune mice. (a) Four-hour treatment of thymocytes with Dex induced a significantly higher increase in the percentage of apoptotic cells of non-autoimmune NON and C57Bl.6 mice than the levels observed in NOD mice. (b) Six-hour treatment of murine splenic cells with Dex induced significantly higher levels of apoptosis in cells of non-autoimmune mice than in cells of NOD mice.

in non-autoimmune strains, namely to the ones observed in the classic GC-susceptible strain, the C57Bl.6 mice (Fig. 2).

Analysis and time course of *c-myc* expression in lymphocytes after GC treatment

Western blot analysis of Myc production after GC treatment of thymocytes and splenic lymphocytes of either diabetes-prone NOD mice or non-autoimmune animals was performed. Lymphocytes obtained from non-autoimmune mice consistently showed a down-regulation of *c-myc* in response to Dex. In contrast, we observed an increase on the levels of Myc protein in lymphocytes, either thymic (Fig. 3a) or splenic cells (Fig. 3b) from NOD mice after *in vitro* treatment of the cells with Dex. This conclusion was confirmed in four independent assays performed with two distinct anti-Myc antibodies. In thymocytes of non-autoimmune mice, *c-myc* expression reached minimum levels 3 hr after *in vitro* GC stimulation, that is 1 hr before the appearance of detectable levels of apoptotic cells. In contrast, thymocytes of NOD mice consistently showed elevated levels of Myc protein, a peak being observed 4½ hr after GC stimulation (Fig. 4). Splenic lymphocytes of non-autoimmune mice showed delayed kinetics of response to GC when compared with thymocytes. Nevertheless, there was also a down-regulation of *c-myc* to its minimum levels about 1 hr before apoptosis detection. Lymphocytes from diabetes-prone NOD mice, similarly to the observations made in thymocytes, consistently showed enhanced levels of Myc, the maximum expression being attained 5 hr after Dex treatment; that is, 1 hr before apoptosis detection (Fig. 5).

Glucocorticoid-induced thymic involution

NOD and C57Bl.6 mice were intraperitoneally injected with 0·2 mg Dex. 48 hr after injection there was a pronounced thymic involution both in NOD mice and the GC-susceptible C57Bl.6 mice as it is documented by changes in the total number of thymic cells (Table 1).

DISCUSSION

We document here that the previously reported apoptosis resistance depicted by NOD mice lymphocytes to GC treatment[10,11] is associated with up-regulation of *c-myc* expressed by increased expression of Myc protein. This change is peculiar to cells of NOD mice as it was absent in the related non-autoimmune NON murine strain, as well as in BALB/c and C57Bl.6 mice. As Myc constitutes a survival signal, high intracellular levels of this protein will signal the cell to live, impairing apoptosis. Thus, elevated levels of Myc protein are likely to be responsible for apoptosis resistance seen in NOD mice lymphocytes treated with GC. Apoptosis is a key cellular event both in the development and in the effective functioning of the immune system, having a crucial role in thymic deletion of autoreactive T cells. Therefore, apoptosis is used to guarantee central self-tolerance of the immune system. Also, in peripheral lymph nodes and spleen similar apoptotic mechanisms are operative in T and B cells. Peripheral deletion by apoptosis is therefore an additional safeguard of the immune system to assure self-tolerance and down-regulation of excessive immune responses. Glucocorticoids (GC) are cell death mediators involved in both central and peripheral immune regulatory mechanisms.[1–5]

Holmberg and coworkers[10,12,13] reported that diabetes-prone NOD mice display defects in several of the pathways leading to apoptosis. NOD mice seem to display enhanced and prolonged immune responses, extended *in vitro* survival,[12] and to be relatively resistant to GC-,[12] interleukin-2 (IL-2) deprivation-,[11] γ-irradiation- and cyclophosphamide-induced apoptosis.[13] The observed GC-induced apoptosis resistance (or delayed apoptotic response) of NOD mice may lead to failure of the mechanism of death by neglect of cells in the thymus, allowing the survival and export of lymphocytes that contribute to the establishment of an autoaggressive T-cell repertoire in the periphery of the immune system. Furthermore, the apoptosis resistance observed in peripheral NOD lympho-

Table 1. Thymic involution induced by Dex* in NOD and C57Bl.6 mice

	Total thymocytes ($\times 10^6$)†	
Strains	Control	Dex
NOD	197	1·2
C57Bl.6	265	3

*Mice were intraperitoneally injected with 0·2 mg Dex.
†Data are representative of a set of four experiments.

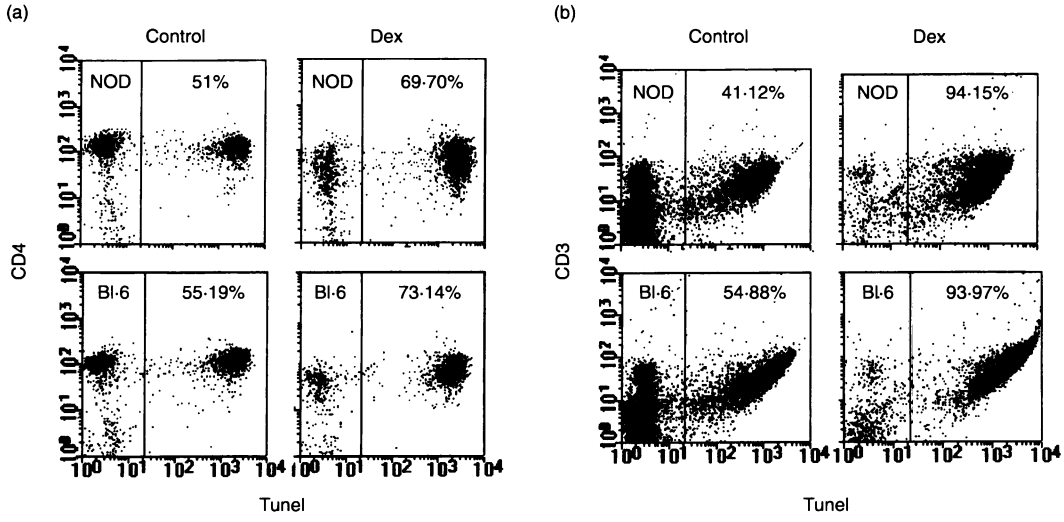

Figure 2. Comparison of the long-term GC response of thymocytes from NOD mice and C57Bl.6 mice. Twelve hours (a) and 24 hr (b) after *in vitro* treatment of thymocytes with Dex, the percentage of apoptosis observed was similar in both strains: diabetes-prone NOD mice and GC-susceptible C57Bl.6 mice.

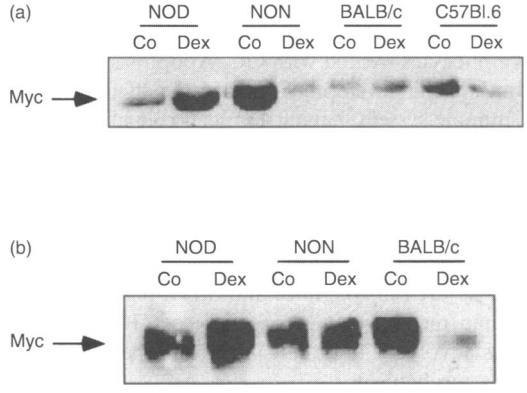

Figure 3. Comparison of *c-myc* expression between normal and diabetes-prone NOD mice. Thymocytes (a) and splenic cells (b) from NOD mice showed a consistent increase in the levels of Myc protein in response to GC, which is in contrast to the decreased levels of this protein observed in non-autoimmune murine strains. Data are representative of four independent assays performed with two distinct anti-Myc antibodies.

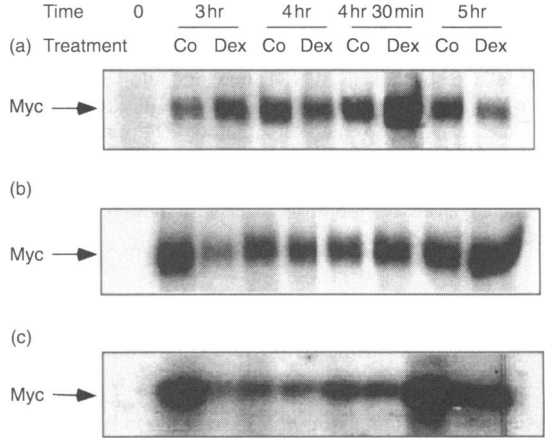

Figure 4. Kinetics of *c-myc* expression after GC treatment of thymocytes from NOD mice and non-autoimmune murine strains. In non-autoimmune NON (b) and BALB/c (c) mice there is a down-regulation of Myc 3 hr after GC stimulation (that is, 1 hr before apoptosis detection). In contrast, NOD thymocytes (a) consistently show elevated levels of Myc. Data are representative of four independent assays performed with two distinct anti-Myc antibodies.

cytes could additionally contribute to the autoimmune pathogenesis observed in these mice, namely through the failure of apoptosis of autoagressive cells that could be induced by regulatory cells or by immunosuppressive agents (glucocorticoids, for instance).

The results presented herein point to a short-term GC resistance of NOD T cells (both in thymic and splenic lymphocytes). In fact, we found that resistance was overcome by prolonged exposure of cells to GC and therefore the phenomenon may, in fact, represent a delay in the response of lymphocytes to GC-induced apoptosis rather than resistance. Indeed, 24-hr *in vitro* exposure of NOD lymphocytes to GC leads to apoptosis of the cells in numbers that are similar to those observed in cells of a classic GC-susceptible strain (C57Bl.6 mice). Also, 48 hr after intraperitoneal injection of GC we observed a similar degree of thymic *in vivo* involution in both NOD and C57Bl.6 murine strains. These findings of ours are in contrast with the observations reported by Leijon *et al.*[12] However, these authors document changes only in the relative proportions of thymic subpopulations of lymphocytes; they did not investigate the net effect of GC on thymic involution. That is, in the total number of thymic lymphocytes; the levels of apoptosis in the thymus after GC treatment were also not reported.[12]

The lethal effect of GC on lymphocytes requires the mediation of their intracellular receptors (GR), which exert a repressive action over gene expression in the nucleus.[14–16] One of the targets of the activated GC-GR complexes is *c-myc*, its expression being down-regulated in response to GC. This effect of GC on *c-myc* expression has been observed in several experimental systems.[15,16,22–25] Studies performed in a human leukaemic cell line, for instance, showed that suppression of

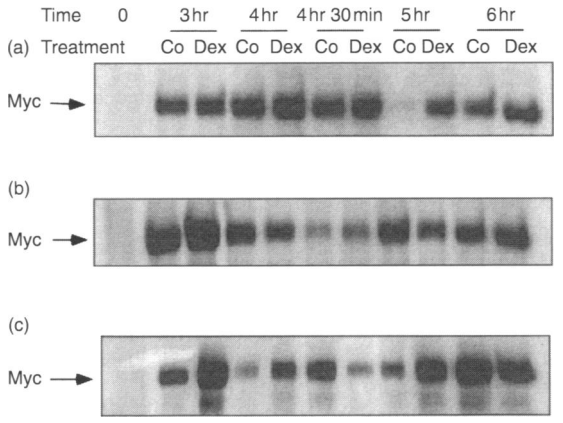

Figure 5. Kinetics of *c-myc* expression after GC treatment of splenic cells from NOD mice and non-autoimmune murine strains. After GC stimulation of cells, there is a down-regulation of Myc to its minimum levels in lymphocytes of non-autoimmune mice (b, c) about 1 hr before detection of apoptotic cells. Lymphocytes from NOD mice (a) consistently show enhanced levels of Myc. Data are representative of four independent assays performed with two distinct anti-Myc antibodies.

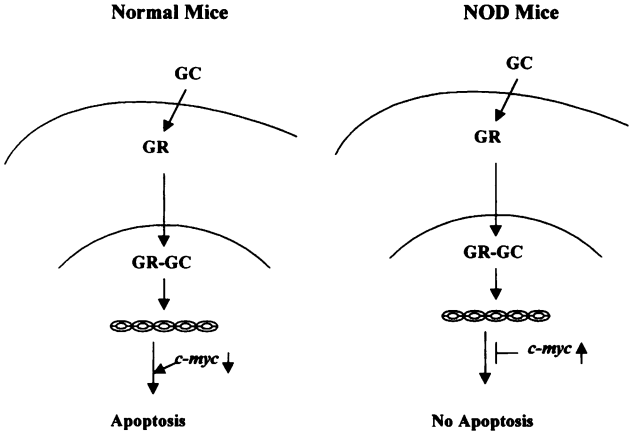

Figure 6. Explanatory model for *c-myc* involvement in the delayed response of NOD mice to GC. In non-autoimmune mice, GC stimulation leads to activation of GC–GR complex, which has a repressive action over gene expression in the nucleus, shutting down survival genes, namely *c-myc*. The down-regulation of *c-myc* promptly allows cell death to occur. As for diabetes-prone NOD mice, the GC response involves a prolonged increase in the levels of Myc protein, which hinders the occurrence of apoptosis of NOD lymphocytes in response to GC.

c-myc mRNA by GC closely correlates with cell lysis and also revealed that sustained expression of *c-myc* provides resistance to GC-induced cell death.[16] Furthermore, the onset of cell death occurred shortly after *c-myc* mRNA and protein reach a minimum. In addition, transfection of antisense *c-myc* oligomers was shown to allow cell death to occur. Similar results were obtained with murine cell lines.[22,26–28] Curiously, T-cell receptor (TCR) ligation, a signal that antagonizes GC-induced cell death, causes elevated expression of *c-myc* in thymocytes.[29]

We found that *c-myc* expression in both thymic and splenic lymphocytes of diabetes-prone NOD mice was enhanced in response to GC, whereas a decrease in Myc protein was observed in the lymphocytes of non-autoimmune mice. This decrease occurred 1 hr before detectable levels of apoptosis were observed. In contrast, 1 hr after apoptosis was seen in lymphocytes of normal mice, cells of NOD mice still failed to down-regulate *c-myc* expression.

We suggest a mechanism to account for the involvement of *c-myc* in the delay of the response of NOD mice cells to GC-induced apoptosis; our proposal is illustrated in Fig. 6 and stated as follows. While in non-autoimmune mice, after GC stimulation, the down-regulation of *c-myc* expression (shutting down a survival gene), owing to the action of GR in the nucleus, allows cell death to occur promptly, in diabetes-prone NOD mice the GC response involves a prolonged increase in the levels of the survival signal represented by the Myc protein, which may account for the delay in apoptosis. Eventually, the levels of Myc protein will decrease and enable cell death to occur. This late decrease would account for the high levels of apoptotic cells of NOD mice that we observed after 12 hr of GC exposure and also for the thymic involution found *in vivo* in NOD mice 48 hr after GC injection. Thus, a paradoxical response of *c-myc* expression to natural GC may constitute a key abnormality in the immune physiology of NOD mouse lymphocytes, leading to autoimmune disease in these animals.

The delivery of a survival signal (*c-myc* up-regulation) in response to apoptotic stimuli ensures the survival of T cells that would otherwise die. This way, thymocytes condemned to die by neglect in the thymus, that is, DP T cells with non-functional or inappropriate TCR (a TCR that does not recognize thymic antigens, for example), would be able to escape to the periphery. These cells potentially include autoreactive T cells, their escape eventually leading to enhanced peripheral autoreactivity, which has in fact been described in NOD mice.[30]

Thus, resistance to GC-induced thymic apoptotic signals, owing to enhanced *c-myc* expression, would then lead to the presence of increased numbers of autoreactive T cells in the periphery of the immune system of NOD mice, this way further predisposing these animals to IDDM development.

ACKNOWLEDGMENTS

The authors are grateful to Professor Carlos Martinez-A for having accepted one of us (T.C.M.) to work in his laboratory during a 6-month period. This investigation was supported by grants from the EC and JNICT (Portuguese Research Council).

REFERENCES

1. Vaux D.L. & Strasser A. (1996) The molecular biology of apoptosis. *Proc Natl Acad Sci USA* **93,** 2239.
2. Krammer P.H., Behrmann I., Daniel P., Dhein J. & Debatin K.-M. (1994) Regulation of apoptosis in the immune system. *Curr Opin Immunol* **6,** 279.
3. Ucker D.S., Hebshi L.D., Blomquist J.F. & Torbett B.E. (1994) Physiological T-cell death: susceptibility is modulated by activation, aging, and transformation, but the mechanism is constant. *Immunol Rev* **142,** 273.
4. Iseki R., Mukai M. & Iwata M. (1991) Regulation of T lymphocyte apoptosis: signals for the antagonism between activation- and glucocorticoid-induced death. *J Immunol* **147,** 4286.
5. Makino S., Kunimoto K., Muraoka Y., Mizushima Y.,

KATAGIRI K. & TOCHINO Y. (1980) Breeding of a non-obese diabetic strain of mice. *Exp Anim* **29,** 1.

6. MILLE B.J., APPEL M.C., O'NEIL J.J. & WICKER L.S. (1988) Both the Lyt2+ and the L3t4+ T cell subsets are required for the transfer of diabetes in nonobese diabetic mice. *J Immunol* **140,** 52.
7. HUTCHINGS P.R., SIMPSON E., O'REILLY L.A., LUND T., WALDMANN H. & COOKE A. (1990) The involvement of Lyt2+ T cells in beta cell destruction. *J Autoimmunity* **3,** 101.
8. YAGI H., MATSUMOTO M., KUNIMOTO K., KAWAGUCHI J., MAKINO S. & HARADA H. (1992) Analysis of the roles of CD4+ and CD8+ T cells in autoimmune diabetes of NOD mice using transfer to NOD athymic nude mice. *Eur J Immunol* **22,** 2387.
9. MARTINS T.C. & ÁGUAS A.P. (1996) Changes in B and T lymphocytes associated with mycobacteria-induced protection of NOD mice from diabetes. *J Autoimmunity* **9,** 501.
10. HOLMBERG D., LEIJON K., PENHA-GONÇALVES C., CILIO C.M., COLUCCI F. & BERGMAN M.-L. (1996) Defective lymphocyte apoptosis—a pathogenic factor in murine IDDM. *The Immunologist* **4,** 128.
11. LEIJON K., HAMMARSTRÖM B. & HOLMBERG D. (1994) Non-obese diabetic (NOD) mice display enhanced immune responses and prolonged survival of lymphoid cells. *Int Immunol* **6,** 339.
12. GARCHON H.J., LUAN J.J., ELOY L., BEDOSSA P. & BACH J.F. (1994) Genetic analysis of immune dysfunction in non-obese diabetic (NOD) mice: mapping of a susceptibility locus close to the Bcl-2 gene correlates with increased resistance of NOD T cells to apoptosis induction. *Eur J Immunol* **24,** 380.
13. COLUCCI F., CILIO C.M., LEJON K., PENHA-GONÇALVES C., BERGMAN M.-L. & HOLMBERG D. (1996) Programmed cell death in the pathogenesis of murine IDDM: resistance to apoptosis induced in lymphocytes by cyclophosphamide. *J Autoimmunity* **9,** 271.
14. HECK S., KULLMANN M., GAST A., PONTA H., RAHMSDORF H.J., HERRLICH P. & CATO A.C.B. (1994) A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *EMBO J* **13,** 4087.
15. HELMBERG A., AUPHAN N., CAELLES C. & KARIN M. (1995) Glucocorticoid-induced apoptosis of human leukemic cells is caused by the repressive function of the glucocorticoid receptor. *EMBO J* **14,** 452.
16. THULASI R., HARBOUR D.V. & THOMPSON E.B. (1993) Suppression of *c-myc* is a critical step in glucocorticoid-induced human leukemic cell lysis. *J Biol Chem* **268,** 18306.
17. KIEFER J., OKRET S., JONDAL M. & MCCONKEY D.J. (1995) Functional glucocorticoid receptor expression is required for cAMP-mediated apoptosis in a human leukemic T cell line. *J Immunol* **155,** 4525.
18. THOMPSON E.B. (1994) Apoptosis and steroid hormones. *Mol Endocrinol* **8,** 665.
19. ROCHA B., LARSSON E.L. & FREITAS A.A. (1984) Effects mof hydroxyurea on concanavalin A-induced T cell proliferation. Depletion of T cell growth factor reactive and producing T lymphocytes. *Scand J Immunol* **19,** 315.
20. TELFORD W.G., KING L.E. & FRAKER P.J. (1994) Rapid quantitation of apoptosis in pure and heterogeneous cell populations using flow cytometry *J. Immunol Meth* **172,** 1.
21. GRAVIELI Y., SHERMAN Y. & BEN-SASSON S.A. (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* **119,** 493.
22. RHEE K., BRESNAHAN W., HIRAI A., HIRAI M. & THOMPSON E.A. (1995) *c-Myc* and cyclin D3 (*CcnD3)* genes are independent targets for glucocorticoid inhibition of lymphoid cell proliferation. *Cancer Res* **55,** 4188.
23. MCCORMACK J.E., PEPE V.H., KENT R.B., DEAN M., MARSHAK-ROTHSTEIN A. & SONEHEIM G.E. (1984) Specific regulation of *c-myc* oncogene expression in a murine B-cell lymphoma. *Proc Natl Acad Sci, USA* **81,** 5546.
24. HIBNER U., BENHAMOU L.E., HAURY M., CAZENAVE P.A. & SARTHOU P. (1993) Signaling of programmed cell death induction in WEHI-231 B lymphoma cells. *Eur J Immunol* **23,** 2821.
25. FISCHER G., KENT S.C., JOSEPH L., GREEN D. & SCOTT D.W. (1994) Lymphoma models for B-cell activation and tolerance. X. Anti-μ-mediated growth arrest and apoptosis of murine B-cell lymphomas is prevented by the stabilization of Myc. *J Exp Med* **179,** 221.
26. FORSTHOEFEL A.M. & THOMPSON E.A. (1987) Glucocorticoid regulation of transcription of the *c-myc* cellular proto-oncogene in P1798 cells. *Mol Encodrinol* **1,** 899.
27. EASTMAN-REKS S.B. & VEDECKIS W. (1986) Glucocorticoid inhibition of *c-myc* and c-Ki-*ras* expression in a mouse lymphoma cell line. *Cancer Res* **46,** 2457.
28. MA T., MAHAJAN P.B. & THOMPSON E.A. (1992) Glucocorticoid regulation of *c-myc* promoter utilization in P1798 T-lymphoma cells. *Mol Endocrinol* **6,** 960.
29. KING L.B. & ASHWELL J.D. (1993) Signaling for death of lymphoid cells. *Curr Opin Immunol* **5,** 368.
30. DELOVITCH T.L. & SINGH B. (1997) The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the NOD. *Immunity* **7,** 727.