Enhancement of anti-tumour immunity in syngeneic mice after MHC class II gene transfection

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Summary The relationship between tumorigenicity and expression of MHC class II molecules in a class II-negative murine leukaemia cell line (LBC) was studied. Analysis of structural DNA sequences encoding MHC class II proteins was performed by Southern blot with DNA isolated from both the original LB tumour and LBC cell line, digested with EcoRI, BamHI and HindIII and hybridised with specific probes for I-A\(^d\) and I-A\(^\beta\) chains. Similar patterns were obtained for LB, LBC and normal BALB/c lymphocytes. In vitro treatment of immunogenicleukaemic cells with IFN-\(\gamma\) (20–100 IU ml\(^{-1}\)) failed to induce the expression of MHC class II antigens in LBC cell line. LBC cells were tri-transfected by a liposome-mediated protocol with I-A\(^d\), I-A\(^\beta\)\(^d\) genes and pSV2neo. Cells were selected for growth in medium containing Geneticin (G418). Surviving transfectants were cloned and three I-A\(^d\) clones, I-A\(^\beta\) clones were obtained after 20 days (LBCT cells). Syngeneic mice inoculated with 1.0 x 10\(^5\) LBCT (I-A\(^d\)) cells failed to develop a tumour, whereas the DT\(_{50}\) of mice injected with 1.0 x 10\(^6\) LBCT cells was three times the value for mice injected with LBC cells (I-A\(^d\)). Furthermore, specific CTL response against tumour cells was significantly enhanced upon priming with irradiated LBC-transfected cells (27±2%) compared with irradiated LBC cells (15±1.5%) in a 4 h \(^{51}\text{Cr}\)-release assay. It is suggested that neoexpression of MHC class II molecules enhances anti-tumour immunity by transforming tumour cells into professional antigen-presenting cells (APCs), which may be used to improve tumour-specific immunity in the autologous host.

Keywords: murine leukaemia; anti-tumour immunity; gene transfection

The term immunosurveillance has been used to describe the concept of natural immunological host resistance against the development of cancer. According to Burnet’s theory, primary immune system function is to recognise and destroy neoplastic cells before they grow and develop into a perceptible tumour (Burnet, 1970).

Tumour cells which display immunogenic epitopes may evade immune detection by lacking the appropriate restriction components (Hammerling et al., 1987). Cytotoxic T cells can recognise intracellular peptide antigens brought to the cell surface in conjunction with major histocompatibility complex (MHC) proteins (Tanaka et al., 1988). As T cells recognise tumour-associated antigens in the context of MHC class I and II molecules, the ability to elicit an anti-tumour response is dependent not only on the presence of tumour-associated antigens but also on an appropriate presentation of these molecules (Ioanides and Whiteside, 1983; Kem et al., 1986). The expression of MHC proteins on tumour cells may be critical for immunological recognition and tumour destruction.

The expression of MHC class II molecules on cells confers on them the ability to act as antigen presenting cells. In some autoimmune diseases, the abnormal expression of class II molecules allows the presentation of self antigens triggering an immune response directly against the aberrant tissue. This is the case for pancreatic \(\beta\)-cells in certain types of diabetes (Bottazo et al., 1985) or for follicular cells in autoimmune thyroiditis (Londei et al., 1984). On the other hand, it has been demonstrated that an enhanced expression of class II molecules in the cells of a murine L1210 lymphoma subpopulation likewise correlated with an increase in immunogenicity and decrease in tumorigenicity (Fuji and Iribe, 1986).

Transfection of MHC genes has been used to study the effect on tumour cell growth in vivo as well as to induce a protective immune response against the tumour itself (Ostrand-Rosenberg et al., 1990; James et al., 1991). Therefore, by inducing neoexpression of MHC class II antigens on neoplastic cells, antigen presentation could be enhanced thus improving anti-tumour immunity in syngeneic hosts.

In previous work we have reported the establishment and characterisation of the LBC cell line (Mongini et al., 1991) derived from the original T-cell leukaemia LB (Ruggiero et al., 1984). LBC cells express the following surface markers: Thy-1, Lyt-2 (CD 8), CD 4\(^{\text{low}}\), CD 25 (IL-2 receptor, \(\alpha\) chain), class I (K\(^d\) and D\(^\beta\)), but not class II (I-A\(^d\), I-E\(^d\)) gene products. Although this cell line is tumorigenic in syngeneic mice, survival rate of mice inoculated with LBC cells is higher when compared with that of mice inoculated with LB cells. Furthermore, when compared with the original non-immunogenic leukaemic cells, LBC induce a weak immune response in their syngeneic host (Mongini et al., 1995).

Taking these findings into account and having demonstrated that LBC cells lack MHC class II antigens, grow aggressively in syngeneic hosts killing the animals in a relatively short time and induce a weak immune response, these cells provide a useful model for studying the relationships of class II molecules expression, immunogenicity and tumour development inter se.

Materials and methods

Mice

Two to four month old normal BALB/c mice were raised in the animal colony of the National Academy of Medicine, Buenos Aires, Argentina, and maintained on Cargill pellets and water \textit{ad libitum}.

Tumour

LB is a T-cell leukaemia, which spontaneously arose in a BALB/c mouse and has been maintained by serial passages in the peritoneal cavity of syngeneic hosts (Ruggiero et al., 1984; Alvarez et al., 1989; Lugasi et al., 1990). LBC is an established cell line derived from LB leukaemia (Mongini et al., 1991). It was maintained in RPMI-1640 (Gibco, Grand...
Island, New York, USA) supplemented with 10% heat inactivated FCS, 2 mM L-glutamine, 20 mM Hepes buffer, 100 µg ml⁻¹ penicillin, 150 µg ml⁻¹ streptomycin and 50 µM 2-mercaptoethanol. Cells were used between passages 120 and 140.

Induction of MHC molecules by treatment with IFNs

LBC cells (3 x 10⁶ ml⁻¹) were incubated for 72 h at 37°C either with 20–1000 IU ml⁻¹ of IFNγ (Genzyme, USA) or 20–1000 IU ml⁻¹ of IFNα+β (Genzyme, USA) and expression of MHC class II molecules analysed by ELISA.

Cell ELISA

This was carried out by the method described previously (Mongini et al., 1995) using 96-well flat bottom microtitre plates (NUNC, Denmark). Briefly, 3 x 10⁵ LBC cells, LBC-transfected cells (LBC-T) or macrophages were seeded into the wells and blocked with 1.5% BSA in TBS buffer (150 mM sodium chloride, 20 mM Tris-HCl; pH 7.4), centrifuged and the supernatant discarded. Cells were fixed with freshly prepared glutaraldehyde (0.25% in TBS) and neutralised with 100 mM glycine–1% BSA. Biotinylated monoclonal antibody clone 39–10–8 (specific for I-Aβ molecules, Pharmingen) was added and incubated at 37°C for 1 h. Avidin–phosphatase-conjugated (Biosys, Institute Pasteur, Paris) was used and the reaction developed by addition of freshly prepared p-nitrophenylphosphate substrate (Sigma Chemical, St Louis, MO, USA). The reaction was stopped with 0.1% sodium hydroxide and read for optical density at 405 nm in an automated ELISA reader (Dynatech, Denmark). Background absorbance was determined in control wells not treated with the first antibody but which had all the other reagents.

Flow cytometry

LBC and LBC-transfected cell suspensions (1.5 x 10⁶) were washed in Hanks’ solution containing 1% BSA and 0.1% sodium azide. Cells were stained with monoclonal antibody MKD6, specific for I-Aβ (Kappler et al., 1981) or with a control isotyping antibody at 4°C for 30 min and, after washing, a second FITC-conjugated antibody was added and cells were left for an additional 30 min at 4°C. Cells were washed, fixed in paraformaldehyde and analysed by flow cytometry on a Becton Dickinson FACScan.

Isolation of eukaryotic DNA and DNA blot hybridisation

DNA was isolated from LB, LBC and normal BALB/c splenocytes according to the method described by Gross-Bellard et al. (1973) with slight modifications. Digested DNA (10 µg) was electrophoresed through a 1% agarose gel and transferred to nylon membrane as described previously (Sambrook et al., 1989). Hybridisation was carried out for 24 h at 60°C in 4 x SSPE, 1% SDS, 0.001% PPI, 0.01 mg ml⁻¹ polyethylenesulphonic acid (Sigma) and 100 µg ml⁻¹ denatured salmon sperm (Sigma) supplemented with random oligo-primed 32P-labelled probes specific for I-Aβ² and I-Aββ² antigens. These probes were kindly provided by L Hood (Steinmetz et al., 1982, 1984). Filters were washed twice at room temperature with 2 x SSC containing 0.5% SDS and twice at 60°C with 0.1 x SSC containing 0.5% SDS. Filters were exposed for 24–48 h at −70°C and developed.

DNA transfection

LBC cells were tri-transfected using Lipofectin according to the procedure described by Dorman (Dorman, 1989), employing pSV2.neo vector DNA, I-Aα² and I-Aββ² genes and selected by growth in medium containing the neomycin analogue G418. Briefly, 2 x 10⁶ LBC cells were co-transfected with 1 µg of pSV2.neo and 10 µg each of I-Aα² and I-Aββ² constructs (Steinmetz et al., 1982, 1984) in a 100 µg of Lipofectin in FCS-free medium solution. Transfectants were selected by growth in medium supplemented with 800 µg ml⁻¹ G418 and resulting clones screened by cell ELISA. The highest I-A-expressing clone, assessed by cell ELISA and by FACS, named LBC-T, was analysed for its biological behaviour and in vivo experiments. Control cells were obtained by transfection of pVS2.neo alone.

Generation of specific anti-tumour cytotoxic cells

Groups of six normal BALB/c mice were inoculated subcutaneously with 10⁶ LBCi cells per mouse or LBC-Ti cells per mouse (LBC or LBC-T cells irradiated with 3000 rads) on days 1, 7 and 14. These mice were injected in the base of the tail with 1 x 10⁶ viable LBCi or LBC-Ti cells a week before the reaction was performed, while 2 x 10⁶ spleen cells ml⁻¹ from normal or immunised BALB/c mice were cultured in RPMI medium for 5 days at 37°C and 4% carbon dioxide in air. Harvested cells were used as effector cells in a ⁵¹Cr-release assay (Coligan et al., 1992).

¹⁷Chromium-release assay

Approximately 1 x 10⁶ tumour target cells were labelled with 100 µCi ⁵¹Cr (Na₂⁵¹CrO₄, Du Pont, NEN Products, Boston, MA, USA). After incubating at 37°C for 45 min, cells were washed three times and plated at 1 x 10⁶ cells 100 µl⁻¹ per well in 96-well round-bottomed culture plates. Effector cells were added to various concentrations, in triplicate, and incubated for 4 h at 37°C. Plates were centrifuged at 200 g for 5 min and 100 µl of cell-free supernatant was harvested from each well and radioactivity determined on a gamma scintillation counter (Alfatron, Buenos Aires). Spontaneous release and maximum release were determined by incubating target cells in medium or in a 2% NP-40 solution respectively. Percentage of specific lysis was calculated as follows:

\[
\text{Percentage of specific lysis} = \left( \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \right) \times 100
\]

Positive controls were generated by co-culturing spleen cells (4.8 x 10⁶ cells per well) obtained from BALB/c mice primed with LBCi or LBC-Ti cells and irradiated Swiss splenocytes (2.4 x 10⁶ cells per well), in a 24-well culture plate. Effector cells were harvested on day 5 and tested against Swiss blast targets in a 4 h ⁵¹Cr-release assay. A cytotoxicity of 10% or more was invariably statistically significant at P < 0.05 and was considered as being positive.

Tumour challenge experiments

BALB/c mice were challenged with 1.0 x 10⁶ or 1.0 x 10⁷ viable LBC and LBC cells. The mortality rate caused by tumour growth was recorded and differences were considered statistically significant at P < 0.05, by log-rank test (Peto et al., 1977). DTₐₙ represented the time at which 50% of challenged mice had died (Mongini et al., 1995).

Statistical analysis

All determinations were done in triplicate and statistical significance was established by Student’s t-test for independent samples. Survival times were analysed with log-rank test (Peto et al., 1977). Differences were considered statistically significant for P < 0.05.

Results

Southern blot analysis of LB and LBC cells

To determine whether LB and LBC cells carry the accurate genetic information for the synthesis of MHC class II molecules (I-Aβ⁰), DNA was isolated from LB, LBC and
normal splenocytes, digested with BamHI, HindIII, EcoRI and analysed by Southern blot (Figure 1). Hybridisation with probes specific for I-Aa\(^d\) and I-Ab\(^d\) genes showed no alterations in LB or LBC cells as compared with controls (normal BALB/c splenocytes).

**Induction of I-A\(^d\) expression on LBC cells by either IFN-\(\alpha+\beta\) or IFN-\(\gamma\) treatments**

In order to determine the possibility that IFN treatments could activate I-A\(^d\) expression on tumour cells, LBC cells were incubated with different concentrations of IFN-\(\alpha+\beta\) or IFN-\(\gamma\) (20–1000 IU ml\(^{-1}\)) and the presence of such molecules was tested by cell ELISA. As Figure 2 shows, it was not possible to induce I-A\(^d\) expression after treatment either with IFN-\(\alpha+\beta\) or IFN-\(\gamma\). However, I-A\(^d\) antigens were enhanced on normal macrophages used as controls.

**I-A\(^d\) gene transfer and expression of LBC cell line**

Neoexpression of I-A\(^d\) molecules was accomplished by transforming LBC cells by liposome-mediated gene transfer and selection with the antibiotic G418. Three clones with different degrees of I-A\(^d\) expression were obtained from the original LBC cell population. The clone expressing the highest level of I-A\(^d\) antigens analysed by cell ELISA and FACS (Figures 3a and 4b) named hereafter LBC\(_1\), was chosen for further experiments designed to evaluate the role of I-A\(^d\) molecules in oncogenesis and host resistance.

**Effect of I-A\(^d\) molecules on tumorigenic properties**

In order to study whether the expression of MHC class II molecules could have any effect on tumour growth, groups of 12 BALB/c mice were inoculated i.p. with 1 x 10\(^6\) or 1 x 10\(^5\) LBCT cells per mouse. Although in vitro LBCT cells had

![Figure 1](image1.png)  
**Figure 1** Southern blot analysis of LB and LBC cells. DNA (10 \(\mu\)g) isolated from LB (lane 1), LBC (lane 2) and normal BALB/c splenocytes (lane 3) were digested with BamHI, HindIII and EcoRI, electrophoresed through a 1% agarose gel and transferred to a nylon membrane as described in Materials and methods. Filters were hybridised with (a) I-Aa\(^d\) and (b) I-Ab\(^d\) specific probes. PM, Molecular weight standard is \(\lambda\) DNA digested with HindIII.

![Figure 2](image2.png)  
**Figure 2** Induction of I-A\(^d\) expression on LBC cells by either IFN-\(\alpha+\beta\) or IFN-\(\gamma\) treatments. LBC cells (3 x 10\(^5\)) were incubated for 72h at 37°C either with 20–1000 IU ml\(^{-1}\) or IFN-\(\gamma\) or 20–1000 IU ml\(^{-1}\) of IFN-\(\alpha+\beta\) and expression of MHC class II molecules was analysed by ELISA as described in Materials and methods. Positive controls were performed by incubating macrophages with 1000 IU ml\(^{-1}\). Results are shown as OD at 405 nm and represent the mean value ± s.d. triplicates. ■, LB cells; ▲, LBC cells; □, macrophages.

![Figure 3](image3.png)  
**Figure 3** Cell ELISA analysis of I-A\(^d\) molecule expression after LBCT I-Aa\(^d\) and I-Ab\(^d\) genes transfer. Approximately 3 x 10\(^5\) LBC, LBCT cells or macrophages were seeded into wells and expression of MHC class II molecules was analysed by ELISA as described in Materials and methods. Results are shown as OD at 405 nm and represent the mean value ± s.d. of triplicates. The absorbance of the negative controls was < 0.005 in all cases. *The highest I-A\(^d\) expressing clone.
identical morphology and growth kinetics to original LBC cells, when \(1 \times 10^3\) LBCT cells were injected they were completely rejected in almost all the mice challenged (10/12 survivors/mice injected, Table I) and the DT50 of mice injected with \(1.0 \times 10^6\) LBCT cells was twice as much as the DT50 for the mice injected with LBC cells (Figure 5). The effect of I-A transfection on tumorigenesis is caused by de novo expression of these molecules rather than the transfection procedure itself since neo-control transfections failed to alter tumorigenic properties.

In vivo induction of anti-tumour specific cytotoxic activity in syngeneic mice

To determine whether immunisation with LBCT cells elicited specific CTL, an in vitro cytotoxicity assay was used. Spleen cells obtained from mice immunised either with LBC or LBCT cells, as described in Materials and methods, were cultured for 5 days and tested for their ability to lyse LBC target cells, using a \(^{51}\)Cr-release assay. As Figure 6 shows, minimal cytolytic activity was detected upon priming with LBC cells. Nevertheless, when transfected cells (LBCT) were used to induce tumour-specific CTL, response was enhanced by 80% (14 ± 1.2% with spleen cells from LBCi primed mice vs 27 ± 2% with splenocytes from LBCTi primed mice). Similar specific cytotoxic response was induced in mice that survived the challenge with transfected cells (LBCT). The response was specific for tumour cells as BALB/c Con A lymphoblasts were not lysed above control levels (data not shown). Spleen cells from non-immunised mice were used as controls for NK activity and invariably rendered less than 2% lysis. Controls for CTL activity were performed in vitro by inducing allogenic CTL [spleenocytes from BALB/c mice immunised in vivo with LBCTi cells and sensitised in vitro with irradiated Swiss splenocytes as shown in Figure 6 Sp (Swiss); error bar).

Discussion

In this work we have reported the induction of MHC class II molecule expression in an LBC tumour cell line (MHC class II negative) and studied the effects on both immunogenicity and tumour cell growth.

Hybridisation with probes specific for I-A\(\alpha\) and I-A\(\beta\) genes showed neither polymorphism nor aberrations, thus ruling out pre-existing gene abnormalities. Consequently, INF\(\gamma\) was used to induce the expression of MHC class II molecules. Induction of I-A molecules on either LB or LBCT cells could not be achieved by INF-\(\gamma\) treatment, in contrast to what has been described for myeloid leukaemias (Lindahl et al., 1974).

These results are in agreement with recent research which showed that INF-\(\gamma\) is incapable of inducing the expression of class II molecules on murine T lymphocytes, at variance with what occurs in human or rat T lymphocytes (Glimcher and Kara, 1992). The lack of an INF-\(\gamma\)-mediated effect on class II expression on either LB or LBCT cells may indicate that alterations have taken place in IFN receptor expression. In our case, the fact that INF-\(\gamma\) treatment inhibited cell proliferation (data not shown) suggests that these cells express intact receptors for this cytokine.

In order to induce neo-expression of I-A\(^\alpha\) molecules, LBC

![Figure 4](image-url)  
**Figure 4** Cytotfluorimetric analysis of IA molecule expression. LBC and LBCT cells were incubated with MKD6 monoclonal antibody or isotype-matched followed by goat anti-mouse FITC. (a) LBCT cells compared with LBC cells. (b) LBCT cells. (c) Negative control.

![Figure 5](image-url)  
**Figure 5** Tumour challenge experiments. Groups of 6 BALB/c mice were injected i.p. with \(10^6\) LB (x), LBC (o) or LBCT (▲) cells per mouse. Data were analysed by log-rank test, and are representative of three independent experiments performed with similar results.

| Table I | LBC and LBCT cell growth in BALB/c mice |
|---------|----------------------------------------|
| Tumour cells | Injection dose | Expression of I-A\(^\alpha\) molecules | No. of survivors/No. of mice infected |
|----------|----------------|--------------------------------------|-----------------------------------|
| LBC      | \(1.0 \times 10^3\) | –                                    | 0/12                              |
| LBCT     | \(1.0 \times 10^5\) | –                                    | 0/12                              |
| LBCT     | \(1.0 \times 10^6\) | +                                    | 10/12*                            |
| LBCT     | \(1.0 \times 10^6\) | +                                    | 2/12*                             |

BALB/c mice were injected intraperitoneally and tumour growth was observed. *Tumour-free mice were observed up to 6 months after cell inoculation.
cells were transfected with I-A^d and I-A^b genes. A low number of clones was obtained and the LBCT cell clone (which expressed the highest level of I-A^d antigens) was chosen to evaluate the role played by I-A^d molecules in onco genesis and host resistance. The number of clones obtained correlated with that described by other authors (James et al., 1991) for experimental models using a triple transfection, similar to the one we employed. In our case three plasmids, two corresponding to each one of the two chains that form the MHC class II molecule, and the remaining one carrying the gene that confers resistance to the antibiotic neomycin, were introduced. The expression of both chains on transfected cells was corroborated by using the monoclonal antibody MKD6, that recognises the complete I-A molecule (Kappler et al., 1981).

LBCT cells (I-A^a) showed identical morphology and in vitro growth kinetics as the parental LBC cells (I-A^a). However, it is worthwhile pointing out that when transfected cells were inoculated in syngeneic mice (1.0 x 10^5 LBCT cells per mouse) they failed to induce tumour growth, in contrast to LBC cells that did so, and the DT_{50} for mice injected with a large inoculum (1.0 x 10^6 LBCT cells per mouse) was three times the value for mice injected with the original cell line. Controls performed transfecting only Neo gene did not express I-A^a molecules and failed to alter tumorigenic properties indicating that the transfection procedure itself is not responsible for preventing tumour growth.

The major role of the immune system in the survival time increase of mice inoculated with LBCT cells was established through studies on specific cytotoxic induction. The significant enhancement of specific CTL response found in mice immunised with irradiated LBCT cells or inoculated with living LBCT cells, when compared with that induced by LBC cells (27 + 2% vs 14 ± 1.2%), indicated that cellular immunity is a crucial mechanism associated with the prevention of tumour growth found in mice challenged with LBCT cells. Humoral immune response induced in syngeneic mice immunised with LBCT cells remained unchanged in comparison with that obtained by the immunisation of mice with LBC cells (data not shown), suggesting that the increase in survival time recorded for mice inoculated with LBCT cells could hardly be caused by effector mechanisms of the immune system involving antibodies, such as the antibody-mediated cellular cytotoxicity or complement fixation.

Strikingly, the weak immunogenicity induced by LBC cell line (Mongini et al., 1995) which derived from a non-immunogenic, aggressive tumour of spontaneous origin (Ruggerio et al., 1984; Alvarez et al., 1989), in common with most human tumours, was considerably enhanced by transfecting LBC cells with I-A^d and I-A^b genes. On the contrary, in the model systems described so far, complete tumour rejection was achieved even though a large amount of neoplastic cells were inoculated after priming with tumour cells transfected with class II molecules (Ostrand-Rosenberg et al., 1990; James et al., 1991; Baskar et al., 1994, 1995). Nevertheless, in these cases, tumour cells were already immunogenic in their syngeneic host, as derived from virus or chemically induced tumours, and the level of immunity generated with live transfected tumour cells might also be induced by non-transfected irradiated tumour cells alone as happens with some tumour cells engineered to secrete cytokines (Moudgil and Sercarz, 1994). In our experimental model, a low number of different epitopes that stimulate anti-tumour response are restricted to a few molecules (Mongini et al., 1994). Therefore, we suggest that the enhancement achieved in cellular immune response seems quantitative rather than qualitative, owing to clonal expansion of specific cytotoxic T lymphocytes and as a result of lymphokines synthesised by helper T lymphocytes that were stimulated directly by the tumour itself.

Concerted action by CD4^+ and CD8^+ T cells in successfully killing tumour cells has been demonstrated by gene transfection of neoplastic cells leading to the release of diverse helper cytokines (Fearon et al., 1990; Pardoll, 1993; Golumbek et al., 1991; Pippin et al., 1994). Although a tumour may succeed in generating a cellular response, ineffective mechanisms of MHC class II restricted presentation may not allow anti-tumour effector cells to activate and reach the critical number required for eradication of the tumour. Recognition of tumour-specific peptides presented on MHC class I molecules by CD8^+ cells, and signals generated by such recognition, may be insufficient to activate precursor CTL cells. One of the additional signals necessary for complete activation of T cells is triggered by lymphokines produced by CD4^+ helper T cells, which usually recognise antigenic peptides presented on MHC class II by antigen presenting cells such as macrophages or dendritic cells. The expression of MHC class II molecules on LBCT cells allows for direct presentation of class II restricted tumour-specific antigens to helper T cells, which could provide the required help for complete activation exerted by cytotoxic T cells. Furthermore, direct participation of CD4^+ lymphocytes in cellular cytotoxic mechanisms restricted by MHC class II molecules (Jacobson et al., 1984; McKisic, et al., 1991; Dadmarz et al., 1995) led us to hypothesise on the intervention of these lymphocytes in conjunction with CD8^+ on direct tumour cell lysis.

Our findings suggest that the induction of class II antigens expression provides an effective method to achieve an immunosurveillance status in cases where tumour cell number is low and metastasis is liable to develop after removal of the primary lesion. Further research may validate such specific immunotherapy as a complement to surgical treatment.

Abbreviations

LB, T cell leukaemia originating in a BALB/c mouse. LBC, cell line derived from LB leukaemia; LBCT, LBC cells transfected with I-A^d genes; LBCi, irradiated LBC cells, LBi irradiated LB cells; LBCTi, irradiated LBCT cells; BSA, bovine serum albumin; TBS, Tris-buffer saline; FCS, fetal calf serum; TBS-T, TBS containing BSA and Tween 20; pNPP, p-nitrophenyl phosphate; ELISA, enzyme-linked immunosorbent assay; SDS, sodium dodecyl
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