UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualifications:-

B.Sc. *B.Sc. (Intercal)*

Immunology C306: Immunobiology

COURSE CODE	:	**IMMNC306**
UNIT VALUE	:	**0.50**
DATE	:	**30-APR-03**
TIME	:	**10.00**
TIME ALLOWED	:	**3 Hours**

TURN OVER

Candidates must answer **Sections A, B and C**. Please answer each section in a separate book.

The fraction of the total marks allocated to each section is as follows:

Section A: 60/180
(**essay**, 1 out of 4)

Section B: 60/180
(**short answers**, 3 out of 6)

Section C: 60/180
(choose **ONE** paper from this section)

TURN OVER

Section A

Answer **ONE** question in this section.

1. Compare and contrast the ways the innate and adaptive immune systems recognise microbes.

2. Discuss the ways in which T cells may "help" an immune response.

3. Why do you think we still do not have an effective vaccine for HIV? Illustrate your answer by comparison to other viral infections for which effective vaccines ARE available

4. Discuss the various immunological mechanisms that may underlie different sorts of "allergy".

Section B

Write short notes on **THREE** of the following:

1. The immunological synapse.

2. Hyperacute rejection.

3. Positive selection.

4. Measuring T cell responses.

5. Antibody-mediated tumour therapy.

6. Maternal tolerance to the foetus.

CONTINUED

Section C

Choose **ONE** of the two papers attached and a) write an abstract which outlines the major findings of the paper, and b) discuss what important further questions this paper raises, and outline briefly the type of experiments you might design to examine these questions further.

a) and b) carry equal marks.

NOTE:

1. **Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen**.

   The following abbreviations are used:

OVA	-	ovalbumin
LPS	-	lipopolysaccharide
BAL	-	bronchio-alveolar lavage (a measure of the number of inflammatory cells in the lung)
APC	-	antigen presenting cells
TLR4d	-	toll-like receptor 4 deficient mice
WT	-	wild type
DLN	-	draining lymph node
BMDC	-	bone marrow derived dendritic cells
PBS	-	phosphate buffered saline

   The Methods section, and all the references have NOT been included.
   NOTE – Figures 1B and Fig 4 are not shown.

2. **Dissociation between autoimmune response and clinical disease after vaccination with dendritic cells**

   Abbreviations:

DC	-	dendritic cells
Ags	-	antigens
ANA	-	anti-nuclear antibodies
PBS	-	phosphate buffered saline

   NZBWF1 mice are a strain of mice which are prone to the development of autoimmune disease.

   The Methods section, and all the references have NOT been included.
   NOTE – Fig 4 is not shown.

TURN OVER

Lipopolysaccharide-enhanced, Toll-like Receptor 4–dependent T Helper Cell Type 2 Responses to Inhaled Antigen

Introduction

Asthma is a pulmonary inflammatory disease believed to be due to aberrant Th2 immune responses to commonly inhaled antigens (1). Only a subset of people exposed to these aeroallergens, however, develop pathological Th2 responses, and this process is not well understood. In particular, the role of adjuvants and the innate immune system in the induction of Th2 responses is unclear.

Respiratory infections have been linked to asthma in both a preventative and facilitating role, implicating Toll-like receptor (TLR) signaling in regulation of Th2-driven airway disease (2). Of particular interest is LPS, a cell wall component of Gram-negative bacteria that is ubiquitous in the environment, including household dusts. LPS activates cells through TLR4 with the accessory proteins CD14 and LPS binding protein (3), signaling through a common adaptor protein MyD88. This results in the transcription of several activation markers including MHC II and B7 molecules and the production of IL-1, IL-12, and TNF-α (3).

The role of endotoxin exposure in asthma development in children has been controversial, with studies indicating either a protective role through Th1 induction or an exacerbating effect on asthma severity (1, 4, 5). It has been speculated that the opposing roles of LPS might be explained by differences in exposure levels (6). However, these studies did not address whether the association of household LPS levels with asthma severity is a result of enhanced allergen sensitization or direct irritant effects of LPS on previously sensitized individuals (4, 6). Our objective was to assess if LPS affects Th2 sensitization to aeroallergens and if the amount of LPS exposure affects the disease phenotype.

It is now clear that Th1 adaptive immune responses require TLR signals (7). However, Th2 priming is thought to occur either as a default pathway in the absence of TLR signaling or by a currently unidentified Th2-type activating receptor(s) (3). Therefore, the role a microbial adjuvant such as LPS plays in Th2 aeroallergen sensitization at the site of natural exposure, namely the lung, is unknown.

To directly address the role of LPS as an adjuvant for Th2 sensitization in the induction of allergic airway responses, we used a murine model of Th2 pulmonary inflammation in which priming occurs after antigen inhalation without the use of alum.

Results

Dose of LPS Determines Type of Immune Response Generated to Inhaled Antigen. We have previously shown that sensitization of mice by exposure to inhaled OVA leads to robust pulmonary Th2 responses (8). To test the role of LPS in these responses, we sensitized mice by intranasal exposure to OVA depleted of contaminating LPS (<0.001 μg) or OVA with a high (100 μg) or low (0.1 μg) dose of LPS. These low and high doses of LPS are analogous to reported endotoxin levels of samples from homes of atopic versus nonatopic children, respectively (5). Mice exposed to LPS-depleted OVA showed no airway inflammatory responses after challenge with inhaled antigen (Fig. 1 A) and had total BAL cell numbers equivalent to PBS controls. In contrast, mice sensitized with OVA containing low dose LPS demonstrated significant increases in total BAL cell numbers as well as lung tissue infiltrates and airway mucus secretion (Fig. 1, A [illegible]). Both airway and tissue infiltrates were dominated by eosinophils, consistent with Th2-mediated inflammation. Draining lymph node (DLN) IL-5 and IL-13 production confirmed the Th2 nature of the inflammatory response (Fig. 1 C). Mice exposed to PBS or low dose LPS alone did not generate pulmonary inflammation after OVA challenge (Fig. 2 A).

As LPS is known to be a potent inducer of IL-12 production from APCs in vitro, it might be expected to preferentially stimulate Th1 responses. Therefore, we tested whether the surprising induction of Th2 responses was a result of the low dose of LPS exposure. Use of a high dose of LPS during intranasal OVA priming resulted in a Th1-associated response dominated by neutrophils and an absence of airway mucus production in the lung (Fig. 1, A and [illegible], reference 10). IFN-γ production from DLNs confirmed the induction of a Th1 response in high dose LPS-exposed mice (Fig. 1 C). Serum antibody isotype patterns in groups sensitized with OVA containing low versus high dose LPS were also consistent with the generation of Th2 (high IgE and IgG1) versus Th1 (high IgG2a) immunity, respectively (Fig. 1 D). Thus, no airway inflammatory response was generated in mice that had been sensitized with LPS-depleted OVA, whereas antigen-specific immune responses were induced in the presence of LPS with low and high doses inducing Th2 or Th1 responses, respectively.

TLR4 Signaling Is Required for Th2 Priming to Inhaled Antigens. The requirement for LPS in the generation of Th2 responses to inhaled antigen was confirmed in C.C3H-*Tlr4*$^{Lps-d}$ mice (13) expressing a nonfunctional TLR4 (TLR4d). When compared with WT, TLR4d mice exposed to OVA in the presence of low dose LPS showed marked reduction in airway inflammation (Fig. 2 A) and DLN Th2 cytokine production (Fig. 3 B, I.N.). We obtained similar results using C3H/HeJ mice. Th1 responses initiated with high dose LPS were similarly abrogated in TLR4d mice (not depicted).

These data support the observation that LPS is required for the development of Th2 (and Th1) responses to inhaled

CONTINUED

antigen. However, because LPS signaling is absent during both sensitization and challenge in TLR4d mice, we next asked at what stage LPS was required (6). To address this question, Th2 cell–dependent OVA-specific antibody secretion was measured. TLR4d mice demonstrated significantly reduced OVA-specific IgG1 and no IgE or IgG2a antibody responses (Fig. 2 B). In addition, there was evidence of a reduced proliferative response in the lung DLN of TLR4d mice as the cellularity after intranasal priming was substantially diminished ($5.9 \pm 1.4 \times 10^6$ in WT vs. $2.3 \pm 0.3 \times 10^6$ cells in TLR4d). Thus, there was evidence of abrogated Th2 priming in TLR4d mice by systemic antibody responses, DLN cellularity, lung inflammation, and cytokine responses, consistent with defective T cell priming in the absence of LPS signaling.

TLR4d Mice Are Capable of Mounting Th2 Responses Using the Adjuvant Aluminum Hydroxide. To confirm that recruitment pathways were intact in the lungs of TLR4d mice, a TLR4 independent mechanism of Th2 priming was used. Alum is a potent Th2 adjuvant that does not contain microbial products and therefore should not involve TLR4 signaling to initiate immune responses. Therefore, TLR4d and WT mice were immunized intraperitoneally with OVA/alum or intranasally with OVA/LPS. 2 wk later, both groups were challenged with inhaled antigen. TLR4d mice were fully capable of initiating Th2 immunity in the presence of a non-TLR4 adjuvant as evidenced by eosinophilic BAL inflammation and Th2 cytokine responses in the lung DLNs (Fig. 3, A and B). Thus, circumventing deficient Th2 priming with the adjuvant alum results in equivalent pulmonary inflammation in TLR4d and WT mice, indicating that lung recruitment of eosinophils and lymphocytes is not impaired in TLR4d mice.

DC IL-12 Production Differs after Exposure to Low and High Doses of LPS. LPS is known to induce both cell surface DC maturation and the production of TNF-α, IL-1, and IL-12 (3). As IL-12 is a potent Th1 skewing cytokine, we hypothesized that differences in IL-12 production following high versus low dose LPS inhalation with OVA might explain the induction of Th1 versus Th2 responses, respectively. To test this, serum IL-12 levels were analyzed. In contrast to mice immunized with low dose LPS OVA, WT mice immunized with high dose LPS OVA had significantly higher levels of serum IL-12 (Fig. 5 A). In vitro evaluation of WT BMDC confirmed that only high dose LPS was capable of inducing IL-12 production, whereas OVA (containing low dose LPS) did not (Fig. 5 B). These data are consistent with the differential inflammatory response observed in vivo (Th1 vs. Th2) and implicate an LPS threshold requirement for IL-12 secretion. Interestingly, TNF-α, a cytokine capable of inducing DC maturation and Th2 sensitization, was unable to induce IL-12 in WT BMDCs. This is consistent with our observations that TNF-α administration during priming was capable of rescuing Th2 responses in TLR4d mice without the induction of Th1 immunity (Fig. 4 A). As expected, no IL-12 was detected from TLR4d serum or BMDCs stimulated with OVA, TNF-α, or LPS.

Discussion

The results presented here support a model of sensitization to inhaled inert proteins that requires LPS and the TLR4 signaling pathway. In addition, the amount of LPS present during sensitization determines whether Th1 or Th2 immunity is observed. Although recent studies in MyD88-deficient mice support a role for TLRs in the generation of Th1 responses to proteins, Th2 responses were shown to be MyD88 independent, suggesting TLR signaling is not important for the induction of Th2 cells (7). However, recent work with MyD88-deficient DCs showed that LPS stimulation induced IL-4 production with normal up-regulation of costimulatory molecules resulting in a Th2 skewing bias (14), suggesting that a MyD88-independent pathway, TIRAP/MAL, is responsible for the observed response. We might speculate that the threshold of induction for these two signaling pathways of TLR4 requires distinct levels of signaling intensities, resulting in differential effects on the adaptive immune response. The results from this study demonstrate the importance of TLR-dependent adjuvants in the induction of Th2 responses and the LPS dose differential of Th1/Th2 activation.

Another study using crystalline OVA in alum intraperitoneally suggested that TLR4-defective mice could not recall Th2-type inflammation to the lung (15). However, the results presented here demonstrate that T cell priming using the adjuvant alum and cell recruitment to the lung are intact in TLR4d mice, as would be expected from an LPS-free, non-TLR–dependent adjuvant such as aluminum hydroxide (Fig. 3 A). This discrepancy may lie in the genetic variation that could occur between the substrains of mice used in their study.

The data reported here may help explain previously observed differences in the response to inhaled protein, where both tolerance and Th2 immunity have been seen (8, 9). It is plausible that these differences are a result of varying levels of LPS contamination and that one reason this protein has been an effective antigen in many asthma models relates to its inherent LPS contamination (16).

Various animal models indicate that exposure to microbial sequences such as LPS can down-regulate Th2 pulmonary responses (17). Epidemiological data in humans support a differential dose model with endotoxin exposure correlated with both increased and decreased incidence of lung disease and severity (1). Our data provide a model to explain these conflicting findings in that OVA exposure in the presence of high dose LPS fails to induce Th2 cells, but instead induces both IL-12 production and a Th1 response. By contrast, low dose LPS is not sufficient to induce Th1 cells but is required to induce Th2 inflammation. In the absence of LPS there is no significant lung response. Thus, different levels of LPS exposure resulting in different Th cell inflammatory responses might explain the discrepancies in human studies. Recently discovered missense mutations in human TLR4 could likewise provide an explanation for the variability in human sensitization to ubiquitous aeroallergens (18).

Respiratory syncytial virus (RSV) infections during childhood have also been identified as a major risk factor for the development of asthma (2). Although RSV is likely to have multiple pathways of influencing asthma, it was recently found that the innate immune response to RSV is mediated by CD14 and TLR4 (19). This raises the question of whether LPS has a unique role in asthma or if other TLR ligands could induce Th2 sensitization.

TURN OVER

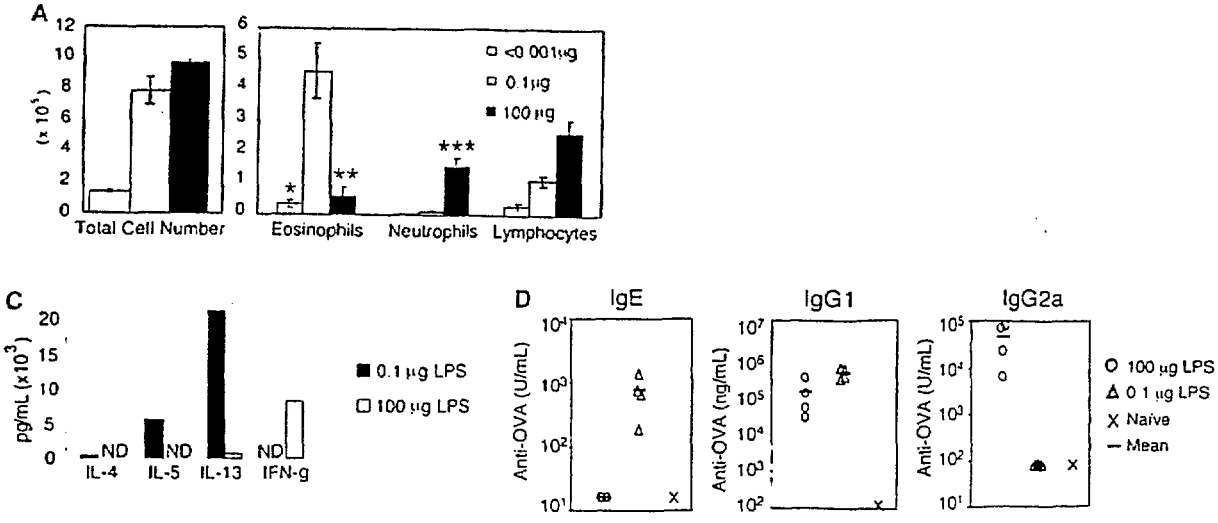

Figure 1. The dose of LPS inhaled with antigen determines the nature of the immune response generated. (A) BAL inflammatory cells of BALB/c mice exposed to LPS-depleted OVA (open bars), OVA with low dose LPS (gray bars), or OVA with high dose *Escherichia coli* LPS (solid bars; Sigma-Aldrich) after challenge. Monocytes constitute the remainder of BAL cells (not depicted). Bars depict the mean ± standard deviation. *, $P < 0.01$ (eosinophils in depleted vs. low LPS groups); **, $P < 0.01$ (eosinophils in high vs. low LPS groups); ***, $P < 0.01$ (number of neutrophils in high vs. low LPS groups). One representative experiment of six is shown. (B) Representative lung sections stained with H&E or PAS at 100×. Arrows indicate areas of peribronchiolar cellular infiltrate (H&E) or positive mucus staining (PAS). (C) Cytokine production from lung draining LNs in low (solid bars) and high (open bars) dose LPS groups. One representative experiment of four is shown. ND, not detectable. (D) Serum antibodies of low (Δ) and high (O) dose LPS groups are compared with pooled sera from naive BALB/c mice (×). Line depicts the mean. $P < 0.05$ (LPS high vs. low dose) for IgG1, IgE, and IgG2a responses.

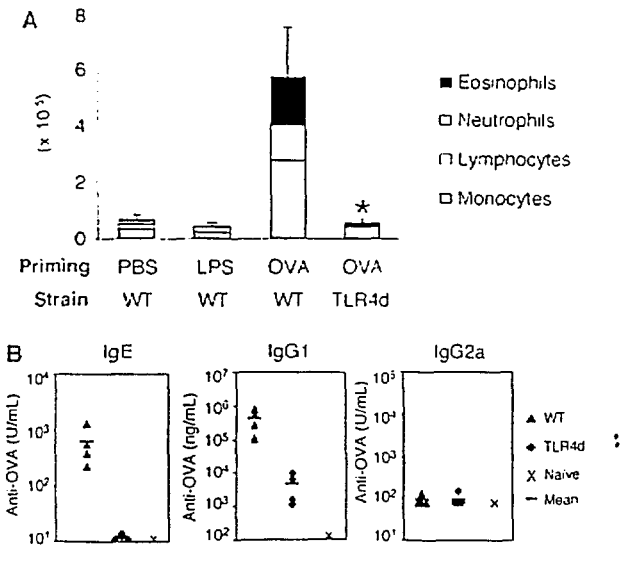

Figure 2. TLR4 signaling is required for Th2 sensitization to inhaled OVA. (A) BAL inflammatory cells of WT or TLR4d mice sensitized intranasally with OVA with low dose LPS (0.1 µg), or WT primed with LPS alone, or PBS on day 21. Total bar height represents total cell number in BAL and error bars are based on total cell numbers. *, $P < 0.04$ (total BAL cell number from TLR4d vs. WT). One representative experiment of six is shown. (B) Serum antibody responses by ELISA on day 21 in WT (▲) and TLR4d (◆) mice compared with pooled naive serum (×). $P < 0.05$ (WT vs. TRL4d) for IgG1 and IgE responses.

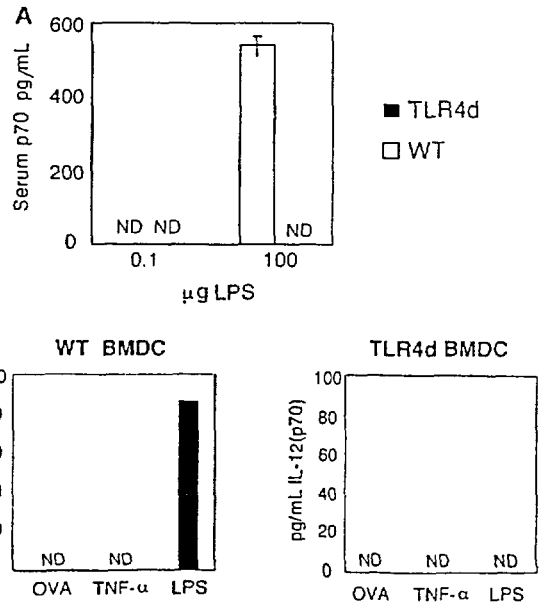

Figure 5. Differential IL-12 production with high and low dose LPS. (A) Serum IL-12 (p70) levels on day 2 of priming with inhaled OVA containing either high (100 µg) or low (0.1 µg) levels of LPS. (B) IL-12 (p70) production from WT or TLR4d BMDCs after stimulation with 100 µg/ml OVA with low dose LPS, 100 ng/ml TNF-α, or high dose (50 ng/ml) LPS for 12 h. ND, not detectable.

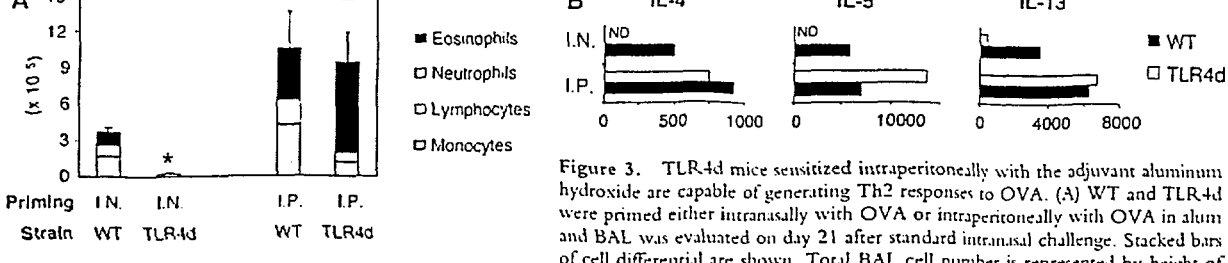

Figure 3. TLR4d mice sensitized intraperitoneally with the adjuvant aluminum hydroxide are capable of generating Th2 responses to OVA. (A) WT and TLR4d were primed either intranasally with OVA or intraperitoneally with OVA in alum and BAL was evaluated on day 21 after standard intranasal challenge. Stacked bars of cell differential are shown. Total BAL cell number is represented by height of stacked bars and standard error is based on total BAL number. *, $P < 0.005$ (intranasally primed TLR4d vs. WT mice). Mice immunized intraperitoneally with alum alone did not respond. (B) Cytokine production in pg/ml from DLN of intranasally or intraperitoneally primed WT (solid bars) or TLR4d (open bars) mice. ND, not detectable. IFN-γ was not detectable from cultures of WT or TLR4d mice primed intranasally or intraperitoneally with OVA containing a low dose of LPS. One representative experiment of two is shown.

CONTINUED

Dissociation Between Autoimmune Response and Clinical Disease After Vaccination with Dendritic Cells

The identification of tumor-associated Ags prompted an effort toward the development of immunotherapeutic strategies. Dendritic cells (DCs)[3] loaded with tumor Ags have been used in patients with melanoma, renal cell carcinoma, colon, breast and ovarian cancer, lymphoma and prostate cancer, with evidence of enhanced T cell immunity and, in some cases, clinical benefit. Whole dying tumor cells represent a source of tumor Ags. DCs that phagocytosed apoptotic tumor cells activate tumor-specific T cells in vitro and in vivo (1–14). Dying tumor cells contain a whole array of tumor Ags in a physical form that ensures their optimal uptake by DCs and the simultaneous activation of MHC class I- and II-restricted T cells. DCs "resurrecting" Ags from dying cells have been suggested to play a role in the initiation of autoimmune diseases. This may impose limitations on antitumor therapies since normal cells and their transformed counterparts share most Ags (15, 16). DC vaccination is accompanied by the activation of autoreactive MHC class I-restricted cytotoxic T cells, with destruction of tissues expressing relevant Ags (15). Different factors, including the turnover of MHC/peptides complexes and the inefficient processing of cell-associated Ags contribute to limit the activation of autoreactive T lymphocytes and to quench the phenomenon (17).

Results

We propagated DCs from bone marrow precursors of BALB/c and NZBW F_1. We irradiated thymocytes and verified by flow cytometry that most cells exposed anionic phospholipids, indicating that they underwent apoptosis (Fig. 1). Furthermore, they excluded PI, indicating that they were still in the early phase of the process. The extent and kinetics of apoptosis of cells from BALB/c or NZBW F_1 mice were similar. We allowed DCs to phagocytose apoptotic cells and retrieved them by magnetic bead sorting of $CD11c^+$ cells. In these conditions, most DCs (consistently >70%) internalized apoptotic thymocytes. Phagocytosis abated at 4°C, i.e., a temperature that does not allow the reorganization of the actin-based cytoskeleton (63 ± 11% at 37°C vs 7 ± 2% at 4°C, $p < 0.01$).

DCs from BALB/c and NZBW F_1 mice expressed before or after phagocytosis similar levels of molecules involved in T cell activation and costimulation, including CD40, CD80, CD86, and MHC class II molecules (Fig. 1 and data not shown). We injected BALB/c or NZBW F_1 mice with DCs that phagocytosed syngeneic apoptotic cells and assessed serological, clinical, or pathologic evidences of autoimmunity. As a control we injected mice with DCs alone or with PBS.

All animals injected with DCs that phagocytosed apoptotic cells developed high titers of autoantibodies (Fig. 2). They developed statistically significant higher titers of ANA than mice immunized with untreated DCs or PBS (NZBW F_1: wk 20 of age, $p < 0.05$ vs DCs and $p < 0.01$ vs PBS-injected animals; wk 30 of age, $p < 0.01$ vs both DC- or PBS-injected animals, Fig. 2, *a* and *b*; BALB/c: wk 20, $p < 0.05$ vs both DC- or PBS-injected animals; wk 30, $p < 0.05$ vs DCs and $p < 0.01$ vs PBS-injected animals, Fig. 2, *c* and *d*). NZBW F_1 mice also developed higher titers of anti-dsDNA (wk 30, $p < 0.05$ vs both DC PBS-injected animals, Fig. 2, *g* and *h*). Fig. 2 also shows that vaccination with DCs that did not phagocytose irradiated cells did not influence ANA and anti-dsDNA Ab titers.

Titers of autoantibodies were similar in 20-wk-old injected BALB/c or NZBW F_1 mice (Fig. 2, *a* and *c*). At wk 30 of age (Fig. 2, *b* and *d*), NZBW F_1 mice titers of ANA and anti-dsDNA Abs were higher than those of BALB/c (Fig. 2, $p < 0.05$). Fig. 2, *e* and *f*, depicts results obtained in representative BALB/c and NZBW F_1 mice. NZBW F_1 mice ANA titers remained higher than control animals and raised steadily in

TURN OVER

boosted animals. In contrast, BALB/c ANA titers dropped to baseline levels. A second self-limiting Ab response developed in BALB/c mice further vaccinated at 24 wk of age.

Fig. 3*a* shows that 12.5% of NZBW F_1 vaccinated with DCs that phagocytosed irradiated cells were dead after wk 28 of age and 50% of animals died by wk 32. At this time point we sacrificed surviving animals and controls. Surviving animals had overt clinical involvement, with a significant increase of the body weight, consistent with anasarcatic state due to end-stage renal disease (average weight ± SD: mice injected with DCs that phagocytosed irradiated cells 43.7 ± 9.4 g, mice injected with DCs alone 31.2 ± 4.3 g, mice injected with PBS 28.4 ± 3.2 g, $p < 0.05$ vs both DC- or PBS-injected animals, Fig. 3, *e* and *f*). BALB/c mice were unaffected (Fig. 3, *b* and *d*). Proteinuria in vaccinated NZBW F_1 mice was the most strikingly affected clinical parameter (Fig. 3*c*). We failed to detect any acceleration in kidney involvement in mice injected with syngeneic DCs alone, which behaved as PBS-injected mice.

BALB/c tissues were healthy. The brain, heart, lung, and liver of NZBW F_1 injected with PBS, DCs alone, and DCs that internalized apoptotic cell were also normal (data not shown). The kidney of NZBW F_1 mice vaccinated with DCs that internalized apoptotic cells exhibited diffuse proliferative glomerulonephritis, with mesangial and capillary hypercellularity, cellular crescents, and fibrinoid necrosis (Fig. 4*a*). Kidney involvement and deposition of immune complexes were confirmed by immunofluorescence and electron microscopy (Fig. 4, *b* and *c*). Healthy NZBW F_1 mice injected with PBS or DCs alone, or BALB/c mice, regardless of the treatment they received, revealed no significant glomerular disease (Fig. 4 and Table I).

Discussion

Stringent constrains regulate the ability of T cells to cause autoimmunity. Two linked events are limiting: 1) the amount and the duration of MHC-restricted epitope presentation in lymph nodes; 2) the ability of DC to process soluble Ags for MHC class I presentation (16, 17). Immature DCs efficiently process cell bodies, presenting derived epitopes in association with MHC molecules. Vaccination of rodents with DCs that phagocytosed in vitro dying tumor cells leads to the recruitment of long-lasting immune responses, endowed with memory and specificity (1, 2, 10, 12). Conversely, DCs that phagocytosed human dying cancer cells prime antineoplastic T lymphocytes in vitro (3, 6, 13, 14). Although the mode of cell death (apoptosis vs necrosis) influences the efficiency of the response in different systems, the cross-presentation of epitopes of dead tumor cells has been substantiated by virtually all studies.

Dead tumor cells share most Ags with their nontransformed counterparts. Immunization of patients with DCs that phagocytosed apoptotic tumor cells is likely to elicit autoimmune responses (18). Our results are consistent with this hypothesis. We vaccinated healthy mice, predisposed or not to the development of autoimmunity, with DCs that phagocytosed apoptotic thymocytes. All vaccinated mice developed autoantibodies. Autoantibodies in normal mice progressively disappeared and did not cause autoimmune disease or tissue damage. In contrast, animals predisposed to autoimmunity developed progressive and eventually lethal organ involvement.

Thymocytes represent a source of syngeneic cells, for which the response to apoptotic stimuli is well-characterized. We used a more stringent vaccination schedule compared with previous reports (1, 10). However, we cannot exclude that different routes or schedule of vaccination could be more efficient in eliciting autoimmunity. Furthermore, the altered gene expression in tumor cells committed to apoptosis and the disease-related immune depression might also influence the outcome of DC vaccination.

Our data are consistent with the lack of clinically evident autoimmunity after vaccination with DCs. In the absence of clinical disease, transient autoantibody responses in vaccinated patients may go undetected. Therefore, the use of DCs that phagocytosed dying tumor cells should be relatively safe. However, extreme caution should be used in the treatment of neoplastic patients with a predisposition to the development of autoimmunity, as suggested by the familial and personal clinical history and by the demonstration of clinical and laboratory features of ongoing autoimmunity.

CONTINUED

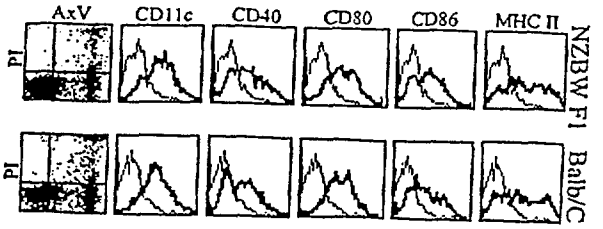

FIGURE 1. DC vaccination. Thymocytes from NZBW F_1 or BALB/c mice were committed to apoptosis by gamma irradiation and analyzed by flow cytometry (*left plots*) after staining with PI (*y*-axis) and FITC-annexin V (*x*-axis). DCs were derived from bone marrow precursors, allowed to phagocytose syngeneic apoptotic cells and injected in vivo (see *Materials and Methods*). Histograms show the expression of membrane molecules from NZBW F_1 and BALB/c DCs, evaluated by flow cytometry immediately before in vivo injection.

Table 1. *Pathological kidney score*

	OM[a]	FM[b]	EM[c]
NZBW F1			
PBS	1/8	2/8	1/8
DCs	1/6	3/6	1/6
DCs + apo[d]	6/7**	7/7**	5/7*
BALB/c			
PBS	0/4	0/4	0/4
DCs	0/4	0/4	0/4
DCs + apo	0/4		1/4

Mice were examined by optical microscopy (OM), fluorescent microscopy (FM) or electron microscopy (EM) by an expert pathologist, and scored from 1 to 4 (normal to increasing severity). The table shows number of mice in each group with a score of 2 or greater/ total number analysed. *, $p < 0.05$, ** $p<0.01$ comparing DC that had phagocytosed irradiated cells, compared to controls.

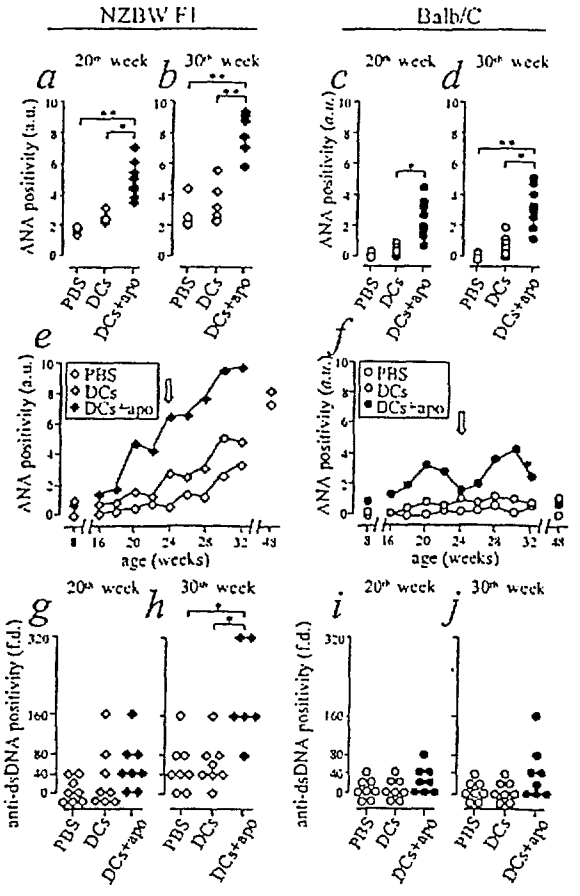

FIGURE 2. Autoantibodies. ANA (arbitrary units) and anti-dsDNA (fold dilutions) Abs were assessed after vaccination of NZBW F_1 mice (diamonds) or BALB/c (circles) with DCs that phagocytosed syngeneic irradiated cells (filled symbols), with DCs alone (gray symbols) or PBS (empty symbols). Data refer to mice at 20 (NZBW F_1, *a* and *g*; BALB/c, *c* and *i*) or 30 wk of age (NZBW F_1, *b* and *h*; BALB/c, *d* and *j*). *e* and *f*, ANA kinetics from representative NZBW F_1 and BALB/c mice. Student's *t* test for ANA and χ^2 test results for anti-dsDNA are: *, $p < 0.05$ and **, $p < 0.01$.

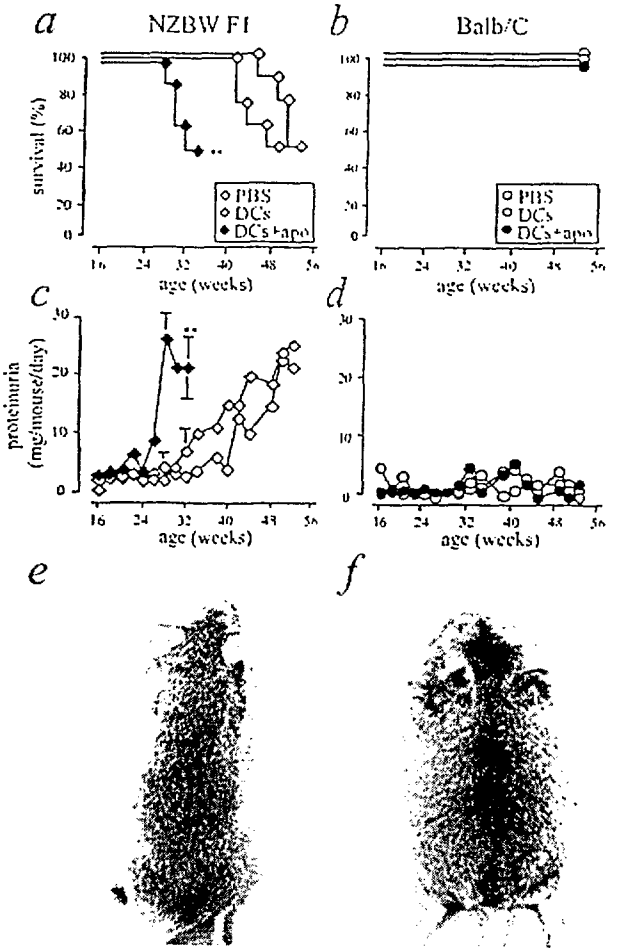

FIGURE 3. Clinical assessment. NZBW F_1 mice (diamonds) or BALB/c (circles) vaccinated with DCs that phagocytosed syngeneic irradiated cells (filled symbols), with DCs alone (gray symbols) or with PBS (empty symbols) were studied. *a* and *b* contain Kaplan-Meyer plots of survival and *c* and *d* proteinuria ± SEM (milligrams per mouse per day, *y*-axis) over time (weeks of age, *x*-axis) from NZBW F_1 and BALB/c, respectively. Fisher's exact and Student's *t* tests results for survival and proteinuria of mice vaccinated with DCs that phagocytosed apoptotic cells compared with controls are: **, $p < 0.01$. *e* and *f*, Pictures of representative 32-wk-old NZBW F_1 mice vaccinated with untreated DCs or DCs that phagocytosed irradiated syngeneic cells, respectively.

END OF PAPER