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The role of microparticles in the generation of immune complexes in murine lupus

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Summary

Systemic lupus erythematosus is a systemic inflammatory disease characterized by antibodies to nuclear molecules in association with immune complex deposition. As shown previously, microparticles (MPs), which are small membrane-bound vesicles released from dying and activated cells, contain nucleic acids and can form immune complexes found in patient blood. To assess the role of MPs in murine lupus, we used flow cytometry to measure the presence of MPs with bound IgG in the blood of MRL-*lpr/lpr* and NZB/W mice. These studies showed much higher numbers of MPs with bound IgG in the blood of MRL-*lpr/lpr* compared to NZB/W mice. Furthermore, these studies showed that antibodies from MRL-*lpr/lpr* mice bound better to MPs from apoptotic cells than those from NZB/W mice. Together, these studies indicate important differences in the serological features of the two strains as reflected by the capacity of antibodies to bind to MPs.

Keywords

autoimmunity; animal models; apoptosis; molecular immunology

1. Introduction

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the production of autoantibodies to nuclear antigens (ANA or antinuclear antibodies) in association with systemic diseases manifestations. Of ANAs, anti-DNA antibodies occur prominently in patients and murine disease models and have diagnostic and prognostic significance. Furthermore, these antibodies can form immune complexes (ICs) that deposit in the kidneys and induce nephritis [1, 2]. In addition to their role in renal disease, ICs can promote pathogenesis by stimulating the production of type 1 interferon by plasmacytoid dendritic cells. This stimulation reflects the intrinsic immune properties of the complexes' nuclear components, especially DNA, and their capacity to stimulate toll-like receptors (TLRs) as well as non-TLR internal nucleic acid sensors [3].

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While the mechanisms of anti-DNA production have been extensively studied, the origin and properties of the nuclear antigens in ICs remain poorly understood. In general, these antigens have been considered to be the products of cell death, with elevated levels in lupus resulting from increased cell death or impaired clearance of dead cell products [4]. As shown in *in vivo* and *in vitro* studies, cell death can lead to extracellular DNA that varies in molecular size and properties. In addition to apoptosis and necrosis, NETosis, which is characterized by the extrusion of high molecular DNA to form an anti-bacterial mesh, produces extracellular DNA either locally or systemically [5, 6]. These considerations suggest that elucidating the antigenic components of complexes is important for understanding the generation of the complexes and devising strategies to block their formation and activity.

As shown in studies on cell free DNA and RNA in the blood, circulating nuclear molecules can exist in either a soluble (or free) or particulate forms. The most abundant particles in blood are called microparticles (MPs) [7]. MPs are small membrane-bound vesicles that are usually 0.1 to 1 micron in diameter and differ from exosomes which are much smaller and originate from the cell interior. While platelets can release MPs during activation, MPs from nucleated cells most likely derive from blebs during apoptosis; blebs are bubble like structures that form on the cell surface and detach by a budding process. The function of blebs is not known, although these structures can contain nuclear as well as cytoplasmic molecules which undergo translocation during apoptosis. MPs have important pro-inflammatory and pro-thrombotic activities and can mediate intercellular communication via their molecular contents [8, 9]. Importantly, blebs are a major source of nuclear autoantigens that are targeted in SLE, with their presence in these structures potentially enhancing immunogenicity [10, 11].

In a previous study, we explored the antigenicity of MPs generated *in vitro* by cell lines undergoing apoptosis [12]. Using flow cytometry (FACS), we showed that murine monoclonal autoantibodies as well as IgG from the plasma of lupus patients can bind particles. These studies showed further that the plasma of lupus patients have dramatically increased numbers of particles expressing IgG, indicative of IC formation, with levels of IgG-positive particles correlating with levels of anti-DNA. Other investigators have reported similar results [13, 14]. Together, these studies raise the possibility that MPs may be an important source of ICs in lupus, differing in dimensions, molecular composition and immunological activity compared to ICs formed from circulating nuclear molecules.

In the current study, we have extended this analysis to murine autoimmunity and investigated the role of MPs in generating circulating ICs in the NZB/W and MRL-*lpr/lpr* lupus models. For this purpose, we used FACS analysis to measure IgG-positive MPs in the plasma from mice collected over time and further investigated the binding of plasma IgG to purified MPs. As results of these studies show, the two strains differ markedly in the number of IgG-positive particles in plasma as well as the ability of plasma IgG to bind to particles of *in vivo* or *in vitro* origin. Whereas MRL-*lpr/lpr* mice, like patients with SLE, consistently have circulating IgG-positive MPs, NZB/W mice have a much lower number of such particles that occur sporadically among individual animals. The plasmas of these strains also differ in their ability to bind to MPs generated *in vitro*. Together, these findings indicate that, despite many similarities in their clinical and serological manifestations, MRL-*lpr/lpr* and NZB/W mice differ in the specificity of autoantibodies as well as the structure of immune complexes.

2. Materials and Methods

2.1. Plasma preparation

BALB/c normal mice and lupus-prone MRL/MpJ-*Fas*^{lpr}/J (MRL-*lpr/lpr*) and NZBWF1/J (NZB/W) strains of female mice were purchased from The Jackson Laboratory, Bar Harbor, ME. The mice were maintained at the Durham Veterans Affairs Medical Center Animal Facility and all procedures were performed in compliance with protocols approved by the Duke University and VA Institutional Review Boards. Every two weeks, blood (up to 250 μ l) was collected from each mouse by sub-mandibular venipuncture in 0.5 ml microtainer tubes (BD Biosciences, San Jose, CA) containing EDTA. Tubes were maintained on ice during the sampling and immediately centrifuged at 1,000 x g for 10 min to sediment cells and separate out the plasma. Plasmas were aliquoted and stored at -20°C long term or at 4°C for up to 5 days for use in assays.

2.2. Purification of IgG

IgG was purified from plasmas using a NabTM Protein A/G Spin Columns kit (Thermo Scientific, Rockford, IL) as per manufacturer's instructions. The amount of IgG in the eluates was measured using a Nanodrop ND-1000 (Nanodrop Technologies, Inc., Wilmington, DE) and 2 or more fractions were pooled if required. Pooled IgG was used to test for binding to MPs.

2.3. Purification of microparticles

Plasma MPs were isolated by differential centrifugation. 500 μ l of pooled plasma from NZB/W, MRL *lpr/lpr* or BALB/c mice were diluted in 500 μ l of PBS. The pool was first centrifuged at 1,000 x g for 10 min and then recentrifuged at 16,000 x g for 30 min to sediment the MPs. The MP pellet was washed in PBS by centrifuging again at 16,000 x g for 30 min. The resulting MP pellet was resuspended in 500 μ l of PBS for use in assays.

Jurkat, THP-1 and HL-60 cells were obtained from the Duke University Comprehensive Cancer Center Cell Culture Facility and were cultured at 37°C and 5% CO_2 in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 20 $\mu\text{g/ml}$ gentamicin (Invitrogen) and 10% fetal bovine serum (Hyclone, Logan, UT). Cells were adjusted to a concentration of 2.5×10^6 cells/ml and treated with 10 μM etoposide for 20 hr to induce apoptosis. Microparticles were obtained by differential centrifugation as described above.

2.4. Determination of microparticle numbers

MPs from mouse plasma and MPs from human cell lines were analyzed by flow cytometry. MP counts were measured using a BD FACScan flow cytometer (BD Biosciences) and detected using light scatter with a side-scatter threshold set to exclude background noise. FlowJo Collectors Edition software (Treestar, Ashland, OR) was used for data acquisition and analyses. MPs were counted during a fixed interval of 30 seconds, corresponding to a sample volume of 30 μ l based on the sample flow rate. Relative MP levels were calculated by dividing the average MP counts from three replicates by the sample volume (30 μ l) and multiplying the result by the dilution factor used in preparing the MP suspension.

MPs were stained to detect surface-bound immunoglobulin using a R-phycoerythrin labeled goat F(ab')₂ anti-mouse IgG reagent (Sigma-Aldrich, St. Louis, MO). Five μ l of plasma was diluted (1:40) in 195 μ l of labeled antibody solution prepared in PBS and incubated for 1 hr at RT. The labeled antibody was diluted to a final concentration of 1:1000. Binding was measured on the flow cytometer and analyzed using the FlowJo software. For quantifying MPs in these experiments, a gate was established around the sub-population of MPs that bound the anti-mouse IgG reagent. The negative controls were MPs from cultures of cells

undergoing apoptosis *in vitro* and stained with the anti-IgG reagent; the reagent did not bind particles non-specifically.

MPs were purified from mouse plasma and cell culture of apoptotic cells as described above. MPs were assayed for binding to monoclonal antibodies that have been characterized extensively [15-17]. QB1 (IgG2b) and PL9-11 (IgG3) which bind DNA and PR1-3 (IgG2a) antibody to a nucleosome complex (H2A-H2B-DNA) were used at concentrations of 10 µg/ml, 60 µg/ml and 40 µg/ml, respectively. Purified IgG from pools of BALB/c, NZB/W and MRL-*lpr/lpr* plasmas were also tested for binding to the *in vivo* as well as *in vitro* purified MPs at a concentration of 200 µg/ml. The plasmas for these pools were terminal bleeds for NZB/W mice at 34 weeks and MRL-*lpr*/mice at 22 weeks. Mouse IgG2a and IgG2b (BD Biosciences) were used as isotype controls. MPs were incubated for 1 hr at RT either with IgG alone or IgG pre-mixed with dsDNA. Antibodies were pre-mixed with 100 µg/ml double-stranded calf thymus DNA (CT-DNA) (Worthington Biochemical, Lakewood, NJ) and incubated for 1 hr at RT to block the DNA binding sites prior to staining MPs. MPs were then stained with a secondary R-phycoerythrin labeled goat F(ab')₂ anti-mouse IgG reagent to detect any bound IgG on the MPs by FACS.

2.5. Anti-DNA ELISA

96-well microtiter plates were coated with 5 µg/ml calf thymus dsDNA (Worthington Biochemical) in sodium citrate buffer and incubated overnight at 4°C. Plates were washed three times with PBS and then incubated with 100 µl of plasma dilution (1:400) prepared in PBS containing 0.5% BSA and 0.05% Tween20. Plates were incubated for 1 hr at room temperature (RT), then washed with PBS. A peroxidase labeled, goat anti-mouse IgG antibody (Sigma-Aldrich) was diluted (1:5000) and used to detect plasma IgG bound to the microtiter plate. The plate was developed for 30 min using a TMB (3,3',5,5'-tetramethylbenzidine) substrate solution in citrate buffer followed by a reagent (10% sulfuric acid) to stop the reaction. Absorbance was measured at 450 nm to determine anti-DNA content.

2.6. Statistical Analyses

MP concentrations measured by flow cytometry and anti-DNA levels measured by ELISA in the lupus-prone and normal mouse plasmas were compared statistically using Student's two-tailed *t*-test for unpaired samples with unequal variances.

3. Results

3.1. Expression of anti-DNA antibody in mouse plasmas

To evaluate the role of MPs in serological events in murine SLE, we first assessed anti-DNA activity by ELISA in plasma from NZB/W, MRL-*lpr/lpr* and BALB/c control mice; for these experiments, plasma were collected at 2 week intervals beginning with mice at the age of 14 weeks; samples were obtained until the time of death. As results in Figure 1 indicate, anti-DNA levels in both strains were much higher than those of control BALB/c mice, with anti-DNA production in MRL-*lpr/lpr* mice occurring earlier than that in NZB/W F1 mice. Anti-DNA levels in MRL-*lpr/lpr* mice were also much higher than those from NZB/W mice.

3.2. Presence of IgG-positive MPs in mouse plasmas

Since previous studies demonstrated the presence of IgG-positive MPs in the plasma of patients with SLE [12, 14], we used FACS analysis to assess the plasmas for MPs with bound IgG, indicative of particle-based ICs. As these studies showed, there were no statistically significant differences (*p*>0.05) in average MP counts between the three groups of age-matched mice over time. Thus, for example, with mice of 22 weeks of age, the

following particle counts were obtained: 7915.7 ± 5924.5 MPs/ μ l [BALB/c], 4960.6 ± 2541.1 MPs/ μ l [MRL-*lpr/lpr*], and 7135 ± 2165.1 MPs/ μ l [NZB/W]. Despite similarity in overall particle numbers among strains, blood of MRL *lpr/lpr* mice consistently showed much higher numbers of MPs with bound IgG (Fig.2A, bottom row; Fig.2B) as compared to the NZB/W and BALB/c plasmas; the difference in the number of IgG-positive particles was observed at all time-points.

3.3. Binding of plasma antibodies to MPs from apoptotic cells

To explore whether antibodies from the NZB/W and MRL-*lpr/lpr* mice can bind particles from nucleated cells, we tested the binding of plasma to MPs generated *in vitro* from THP-1, HL-60 and Jurkat cell lines induced to undergo apoptosis by treatment with etoposide. The antigenicity of the *in vitro* generated MPs was verified using two monoclonal anti-DNA antibodies (PL9-11, from an MRL/Mp- +/+ mouse and QB1, from A.SW mice)[15, 17](Fig. 3A). As these studies showed, whereas an MRL-*lpr/lpr* plasma pool contains IgG that binds MPs from cell lines, a pool of NZB/W lupus plasma did not show IgG binding in these experiments although an occasional plasma obtained at other time points did contain IgG-positive particles. The specificity of this binding was evaluated by pre-incubating the antibody or plasma preparation with purified calf thymus DNA (CT DNA). Binding of QB1 was significantly reduced by pre-incubation in CT-DNA (Fig.3A) whereas the binding of PL9-11 showed less inhibition. Furthermore, pre-incubating the MRL-*lpr/lpr* plasma with CT DNA reduced the IgG binding to MPs (Fig.3B). These results indicate that MPs display DNA (and likely other nucleosomal antigens) which can be bound by anti-DNA monoclonal antibodies as well as antibodies from MRL-*lpr/lpr*.

3.4. MPs from mouse plasma are antigenic and bind IgG

While these studies suggest that the specificity differences in the antibodies in the two strains account for the differences in the levels of IgG-positive particles, they do not exclude differences in antigenicity of the circulating particles; these differences could also be relevant to immunogenicity. We therefore investigated the antigenicity of MPs purified from plasma from BALB/c and NZB/W mice. For this purpose, we tested a panel of anti-DNA and anti-nucleosome monoclonal antibodies as well as purified IgG from all three strains of mice. The monoclonal antibodies were specific for DNA (PL9-11, QB1) and nucleosome (PR1-3, from an MRL-*lpr/lpr* mouse) [15-17]. Previous studies demonstrated that these antibodies bind particles generated *in vitro* from apoptotic cells [12]. In these experiments, we did not assess particles from MRL-*lpr/lpr* plasmas since they already had bound antibody which could occupy antigenic sites.

As shown in these studies, the QB1 anti-DNA and the PR1-3 anti-nucleosome monoclonal antibodies can bind MPs purified from BALB/c and NZB/W plasma, consistent with the display of DNA and other nucleosomal antigens that can be translocated to particles during apoptosis [18]. As expected, binding of QB1 was DNA-dependent as the FACS signal was reduced by prior incubation with CT DNA; this treatment did not affect the binding of PR1-3. In addition to binding monoclonal antibodies, particles also bound to purified IgG from MRL *lpr/lpr* plasma (Fig.4) whereas neither NZB/W nor BALB/c plasma IgG bound significantly to the MPs. The IgG signal of the plasma IgG was only slightly reduced by pre-incubation with CT-DNA, suggesting the presence of antibodies other than anti-DNA that could bind particles. These results indicate that particles from NZB/W mice can react to antinuclear antibodies and suggest that the paucity of IgG-positive particles in NZB/W plasma results from the specificity profile of the antibodies.

4. Discussion

The results of these studies provide a new perspective on the generation of immune complexes in murine lupus and demonstrate important differences among strains in the role of microparticles as a source of immune complexes. Thus, as shown by FACS analysis, plasma from the MRL-*lpr/lpr* strain had significantly higher number of IgG-positive MPs compared to the NZB/W strain although the total number of MPs did not differ significantly. These findings are similar to studies on MPs in human lupus which indicated that, while total number of MPs may not be greater than those in normal controls, the number of particles with IgG is significantly increased [12, 14]. In this regard, the studies on patients indicated differences in the number of IgG-positive particles among individuals, a result not unexpected in view of the clinical and serological heterogeneity of patients and potential role of disease activity in affecting particle number.

The most striking finding in this study is the marked difference among the two strains of lupus mice in the number of IgG-positive particles despite their common expression of anti-DNA (Figure 1). There are at least two potential explanations for these findings. Thus, although both strains may express anti-DNA and other antinuclear antibodies, these antibodies may differ in fine specificity, with MRL-*lpr/lpr*, but not NZB/W antibodies, able to bind antigenic sites on particles. As previously shown, binding to particles is a property of some but not all monoclonal anti-DNA and anti-nucleosomal antibodies as well as patient plasma [12]. These differences may relate to the exposure of particular epitopes on the particle surface. In this regard, the difference in binding of MPs by antibodies was evident with plasma as well as purified IgG (Figure 4). In titration experiments, the binding by MRL-*lpr/lpr* IgG to MPs was dose-dependent and demonstrable at concentrations as low as 12.5 µg/ml (data not shown). These experiments indicate that the difference among strains in plasma binding to particles does not simply reflect IgG levels in the plasma. The second explanation relates to potential differences in the antigenicity of particles produced in the two strains, with MRL-*lpr/lpr* mice producing particles that have a greater display of nuclear and possibly other antigens than the corresponding particles from NZB/W mice. Differences in the origin of particles (e.g., nucleated cells vs platelets) could also result in differences in antigenicity.

Results of the studies to distinguish antigenicity of MPs from the different strains of mice indicate that the MPs from NZB/W and BALB/c mice are similar (Fig.4) to each other as well as to particles generated *in vitro* from cells undergoing apoptosis. Since particles from MRL-*lpr/lpr* mice have bound IgG and the MRL-*lpr/lpr* plasma IgG can bind particles, MRL-*lpr/lpr* particles are also likely to be antigenic.

Formally, it is possible that the IgG on MRL-*lpr/lpr* MPs results from another mechanism (e.g., binding of preformed immune complexes to circulating molecules via Fc receptors). It is also possible that at least some of the antibodies binding particles react with membrane or cytoplasmic components as opposed to nuclear molecules although nuclear antigens may be present in the particles. As previous studies have shown, particle membranes are permeable as indicated by binding of propidium iodide [19]. Antibodies may therefore have access to molecules on the inside as well as outside of particles. Proteomic studies have demonstrated an abundance of particle components; theoretically, any of these molecules could be an autoantigen and contribute to particle binding [20, 21].

While the origin of circulating particles and their antigenicity require much further study, the current studies provide a new perspective on the serology of murine lupus and the heterogeneity of anti-DNA production. Importantly, while NZB/W plasma had significant levels of anti-DNA antibodies, nevertheless, these plasmas had very limited or no binding to

particles generated *in vitro* although these particles bound readily to monoclonal anti-DNA and antinucleosomal antibodies as well as plasma from MRL-*lpr/lpr* mice and lupus patients. Studies on the specificity of anti-DNA antibodies have demonstrated marked differences in their binding to a variety of purified DNA antigens (natural and synthetic) indicative of differences in fine specificity including interaction with sequential determinants [22]. These antibodies can differ in avidity and nephritogenicity although, in general, anti-DNA production in murine as well as human SLE has been considered to be both diverse and polyclonal. Nevertheless, our studies indicate a significant lack of antibodies in NZB/W plasma that can bind particles despite the DNA content of particles.

Since blebs (from which MPs likely arise) may play a role in ANA production because of their content of nuclear molecules, these findings suggest that strains may differ in the extent to which blebs (and their antigens) drive autoantibody production. It is possible that, in NZB/W mice, a source of nuclear material other than blebs drives autoantibody production. It is of interest in this regard that MRL-*lpr/lpr* mice have much greater anti-Sm responses than NZB/W mice although detection of these responses may differ depending on assay technique [23, 24]. Sm and RNP antigens also occur in blebs, suggesting that, in some mice (as well as patients), the serological profile may reflect the nature of the driving antigen and the extent to which blebs and MPs serve as immunogens [11].

Finally, differences in the binding of antibodies to MPs may relate to clinical disease manifestations among strains. For example, in addition to nephritis, MRL-*lpr/lpr* mice have evidence of arthritis, vasculitis and Sjogren's-like infiltrates; in humans, MPs have been associated with these manifestations [7]. These clinical features are less prominent in NZB/W mice [25, 26]. Another clinical manifestation where MPs immune complexes may play a role is nephritis since particles are a potential source of immune complexes although this role has not been verified. It is of interest that, while immune complex formation is an important feature of patients and murine models, the antibodies responsible for nephritis and mechanisms of renal IC deposition in nephritis have not been clearly established [27]. Furthermore, in NZB/W mice, genetic studies have demonstrated an important role of antibodies to gp70 in nephritis [28, 29].

It is of interest in this regard, that a recent study of lupus mice (NZB/W, MRL-*lpr/lpr*, BXSB, NZM2410) showed important differences in the serological profile of various strains as shown using an array technique as well as ELISA assays for both IgG and IgM responses [30]. Of note, MRL-*lpr/lpr* mice had the highest levels of ANA and anti-glomerular antibodies.

5. Conclusions

Together these findings suggest that lupus mice, like patients, may differ in the fine specificity of the serological responses in a way that impacts on disease manifestations and the formation of ICs that either drive cytokine production or deposit in the tissue. Importantly, to the extent that MPs form pathogenic ICs, the paucity of IgG-positive MPs in the NZB/W mice, coupled with the at best limited binding of NZB/W plasma to bind to MPs, could suggest that the anti-DNA antibodies may not contribute as much to nephritis in comparison to other autoantibodies (i.e., anti-gp70). Studies are therefore in progress to determine the specificity of anti-DNA binding particles and their ability to promote nephritis.

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Abbreviations

MPs	microparticles
ANA	antinuclear antibodies
IC	immune complex
SLE	systemic lupus erythematosus

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Highlights

- Blood of lupus mice contain microparticles with IgG as shown by flow cytometry.
- MRL-*lpr/lpr* and NZB/W mice differ in levels of IgG positive particles in blood.
- MRL-*lpr/lpr* antibodies have higher binding to microparticles than NZB/W antibodies.
- Fine specificity of lupus autoantibodies influences microparticle binding.
- The structure of immune complexes differs among strains of lupus mice.

Figure 1. Anti-DNA levels in mouse plasmas

Nine MRL-*lpr/lpr*, 9 NZB/W and 5 BALB/c plasmas were tested by ELISA for anti-DNA at 2-week intervals. Antibody assays were performed as described in Materials and Methods. The bars indicate anti-DNA from NZB/W (hatched), MRL-*lpr/lpr* (grey) and BALB/c control (black) plasmas. The asterisks denote mean values significantly different (Student's t-Test) compared to BALB/c levels. Each sample was tested in triplicate and the experiment was repeated three times.

Figure 2. The presence of MPs with bound IgG

A) Plasma MPs from individual 22-week old MRL-*lpr/lpr*, 26-week old NZB/W and control BALB/c mice were stained with phycoerythrin-labeled goat anti-mouse IgG (γ -chain specific) and assessed by FACS. The grey peaks denote unstained MPs and the solid lines indicate MPs stained with the anti-IgG reagent. The horizontal gate excludes any background fluorescence of MPs and indicates the IgG-positive MPs that bind the reagent strongly. The results are representative of four replicates. B) IgG binding to plasma MPs in longitudinal bloods. Nine MRL-*lpr/lpr*, 9 NZB/W and 5 BALB/c plasmas collected at two week intervals were analyzed by FACS using a phycoerythrin-labeled goat F(ab)₂ anti-mouse IgG reagent to detect MPs with bound IgG. The plot shows the percent mean IgG-positive MPs at each time point in MRL-*lpr/lpr* (gray bars), NZB/W (hatched bars) and BALB/c (black bar) mouse plasmas. The asterisks denote percent mean IgG-positive MP values that differ significantly compared to BALB/c MPs by Student's t-Test. Each sample was tested in duplicate and the experiment was repeated twice.

Figure 3. The binding of monoclonal antibodies and plasma to MPs from apoptotic cells

A) The binding of monoclonal antibodies to MPs generated *in vitro*. Columns show FACS staining of MPs from apoptotic HL-60, Jurkat and THP-1 cells in the absence (MPs only) or presence of QB1 (anti-DNA) and PR1-3 (anti-nucleosome). Dotted line shows binding following pre-incubation with dsDNA (dotted line). B) The binding of mouse plasmas to MPs. As these data indicate, MRL-*lpr/lpr* plasma IgG binds MPs (solid line). This binding can be reduced by pre-incubation with CT-DNA (dotted line). Under these conditions, BALB/c and NZB/W plasmas did not show significant binding to MPs. The horizontal gates indicate the proportion of MPs that stain due to IgG binding (solid lines only). These results are representative of four replicates.

Figure 4. Binding of antibodies to MPs isolated from plasma of BALB/c and NZB/W mice
 MPs isolated from BALB/c and NZB/W plasmas were incubated with monoclonal antibodies (QB1, PR1-3) or purified IgG from BALB/c, NZB/W and MRL-*lpr/lpr* plasmas at 200 $\mu\text{g/ml}$ and binding assessed by FACS. The dotted line indicates background fluorescence from the PE-labeled anti-mouse IgG and the solid line indicates staining due to monoclonal antibodies or purified plasma IgG. The control panels demonstrate the specificity of antibody binding. As these data indicate, QB1 shows higher binding to both BALB/c and NZB/W MPs (thick solid peak) compared to secondary antibody alone (thin solid line) and unstained MPs (grey peak). Furthermore, incubation with CT-DNA (10 $\mu\text{g/ml}$) reduced binding of QB1 binding (broken line) to both types of plasma MPs. These results are representative of three replicates.