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Technical note

Blocking glomerular immunoglobulin deposits in a mouse model of lupus nephritis on indirect immunofluorescence with the use of Fab fragments

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ABSTRACT

One of the most characterized models of murine lupus nephritis is the [NZB × NZW] F1 female hybrid. Extended glomerular IgG deposits may pose an obstacle in studying molecules of interest via indirect immunofluorescence due to secondary antibodies non-specific binding to deposited IgG molecules. Application of Fab fragments may mitigate non-specific interactions in this mouse model. Specifically we provide evidence that blocking paratopic interactions of secondary antibodies with indigenous glomerular IgG deposits is possible. However the blocking effect seems to be related to the species used for secondary antibody production. Increased secondary antibody host species homology with the mouse could make blocking of non-specific binding via the use of Fab fragments impossible in this mouse model.

1. Introduction

One of the best characterized models of murine lupus nephritis is the [NZB × NZW] F1 female hybrid (Srividya Subramanian, 2007). These mice develop severe glomerulonephritis by the age of 5–6 months (Seegal et al., 1969). Immune complexes containing IgG immunoglobulins are deposited within the glomerulus in subendothelial, subepithelial and mesangial areas as well as in tubular basement membranes (Hurd and Ziff, 1977; Sugisaki and Takase, 1991). The presence of immune complexes makes the study of epitopes of interest via indirect immunofluorescence difficult or even impossible. This is due to paratopic interactions of secondary antibodies within deposited immune complexes. The present technical note shows that blocking of paratopic interactions is possible with the use of Fab fragments. However inter-species homology could possibly determine the degree of paratopic interactions and hence the efficacy of utilizing Fab fragments as a blocking tool.

2. Laboratory animals

Seven months old NZBW/F1 and C57BL/6 mice were obtained from the Institute of Molecular Biology and Biotechnology, Foundation Research and Technology, Crete, Greece. C57BL/6 mice served as controls. Experiments were carried out in accordance to the current legislation on animal experiments in the European Union and approved by our institution's Safety and Ethics Committee for Animal Research. Mice had free access to water and a standard laboratory diet, and were housed in a room with constant temperature and humidity, and a 12-h dark/light cycle. Anaesthetized animals using pentobarbital intra-peritoneally, were sacrificed via cervical dislocation.

Abbreviations: NZBW/F1, [NZB × NZW] F1 female hybrid; Fab fragments, Fragment antigen binding.

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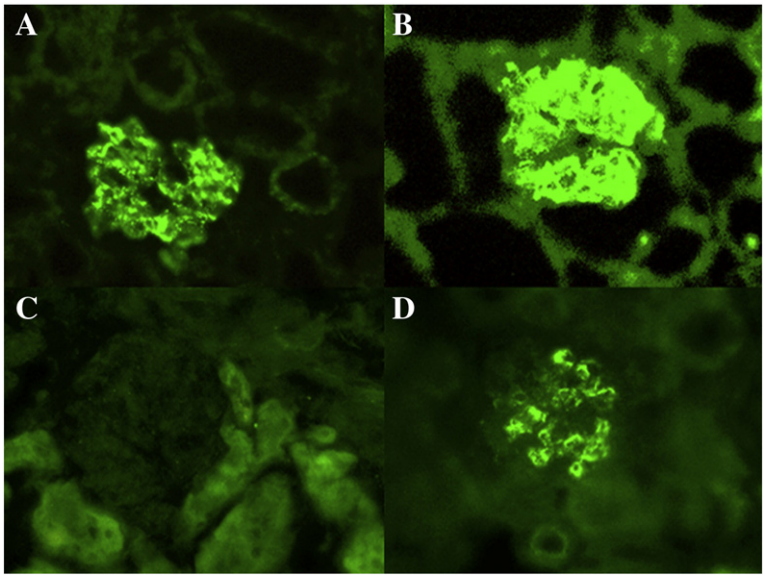

Fig. 1. The effect of Fab fragments as a blocking tool in the application of different secondary antibodies and incubation conditions. A). Goat anti-rabbit IgG (vs H + L) Alexa Fluor 488 and blocking with 5% NGS for 60 min at ambient temperature. B) Goat anti-rat IgG (vs H + L) Alexa Fluor 488 and blocking with 5% NGS for 60 min at ambient temperature. C) Goat anti-rabbit IgG (vs H + L) Alexa Fluor 488 and blocking with 5% NGS for 60 min at ambient temperature. Blocking with 0.1 mg/ml goat anti-mouse IgG Fab (vs H + L) fragments for 90 min at ambient temperature. D) Goat anti-rat IgG (vs H + L) Alexa Fluor 488 and blocking with 5% NGS for 60 min at ambient temperature. Blocking with 0.5 mg/ml goat anti-mouse IgG Fab (vs H + L) fragments for 200 min at ambient temperature.

3. Materials and methods

3.1. Kidney tissue processing

The capsule was removed and both kidneys were sectioned in the sagittal and transverse plane. Each piece was embedded in O.C.T (Sakura) and then was acutely frozen in liquid nitrogen cooled isopentane (Sigma Aldrich). Renal tissue was then stored at −80 °C for further processing.

3.2. Immuno-histochemistry protocol

Five μm thick kidney tissue cryosections were cut at −20 °C. Cryosections were mounted on fish gelatin (Sigma) coated slides. Freshly made 4% paraformaldehyde (Sigma) in Phosphate Buffered Saline (PBS) solution, pH = 7.2, was applied on tissue sections for 13 min. Triton X-100 0.1% in PBS, pH = 7.2, was applied for 10 min. Blocking was performed with 5% normal goat serum (Gibco) in PBS, pH = 7.2, for 60 min at ambient

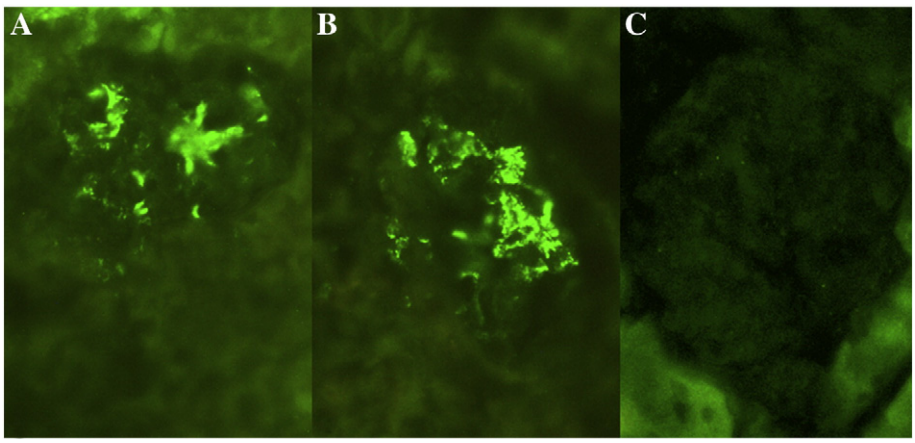

Fig. 2. The use of Fab fragments as a blocking tool at concentrations lower than 0.1 mg/ml when applying goat-anti rabbit IgG secondary antibody. A) Goat anti-rabbit IgG (vs H + L) Alexa Fluor 488 and blocking with 5% NGS for 60 min at ambient temperature. Blocking with 0.001 mg/ml goat anti-mouse IgG Fab (vs H + L) fragments for 90 min at ambient temperature. B) Goat anti-rabbit IgG (vs H + L) Alexa Fluor 488 and blocking with 5% NGS for 60 min at ambient temperature. Blocking with 0.01 mg/ml goat anti-mouse IgG Fab (vs H + L) fragments for 90 min at ambient temperature. C) Goat anti-rabbit IgG (vs H + L) Alexa Fluor 488 and blocking with 5% NGS for 60 min at ambient temperature. Blocking with 0.1 mg/ml goat anti-mouse IgG Fab (vs H + L) fragments for 90 min at ambient temperature.