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Signal transducer and activation of transcription 6 (STAT6) regulates T helper type 1 (Th1) and Th17 nephritogenic immunity in experimental crescentic glomerulonephritis

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Summary

Experimental crescentic glomerulonephritis is driven by systemic cellular immune responses. A pathogenic role for T helper type 1 (Th1) and Th17 cells is well established. T-bet, a key transcription factor required for Th1 lineage commitment, and retinoic acid-related orphan receptor-γt (Rorγt), a key Th17 transcription factor, are required for full expression of disease. Similarly, several Th1- and Th17-associated cytokines have been implicated in disease augmentation. The role of Th2 cells in the disease is less clear, although Th2-associated cytokines, interleukin (IL)-4 and IL-10, are protective. We sought to determine the role of signal transducer and activation of transcription 6 (STAT6), a key regulator of Th2 responses, in experimental crescentic glomerulonephritis. Compared to wild-type mice, histological and functional renal injury was enhanced significantly in STAT6$^{-/-}$ mice 21 days after administration of sheep anti-mouse glomerular basement membrane globulin. Consistent with the enhanced renal injury, both Th1 and Th17 nephritogenic immune responses were increased in STAT6$^{-/-}$ mice. Conversely, production of IL-5, a key Th2-associated cytokine, was decreased significantly in STAT6$^{-/-}$ mice. Early in the disease process systemic mRNA expression of T-bet and Rorγ was increased in STAT6$^{-/-}$ mice. We conclude that STAT6 is required for attenuation of Th1 and Th17 nephritogenic immune responses and protection from crescentic glomerulonephritis.

Keywords: glomerulonephritis, renal immunology/disease, T cells

Introduction

Glomerulonephritis (GN) is a common cause of renal disease, including end-stage renal failure. Experimental crescentic GN is the murine homologue of rapidly progressive GN, the most severe form of GN. Severe injury in this model is mediated by cellular immunity and CD4$^+$ T cells are key components of renal injury [1,2].

Upon activation, naive CD4$^+$ cells tend to differentiate into subsets (T helper cells – Th1, Th2 and Th17) that engage immune effectors in different ways. In proliferative forms of GN, T cells direct adaptive immune responses that drive glomerular disease, but also, in rapidly progressive GN, CD4$^+$ cells themselves accumulate in glomeruli as effectors. These effector T helper cells activate innate immune effector cells, predominantly neutrophils and macrophages, which activate and damage intrinsic renal cells. While humoral immunity influences the patterns and severity of some forms of GN, in this model severe renal injury is driven by cell-mediated immunity [3] and occurs independently of autologous antibodies [4]. There is evidence that both Th1 [5] and Th17 [6] responses are pathogenic in experimental crescentic GN. Deficiencies in the key transcription factors, T-bet for Th1 cells [7] and retinoic acid-related orphan receptor-γt (Rorγt) for Th17 cells [8], result in significantly attenuated renal injury.

Traditionally, Th2 cells have been considered essential for host protection from parasitic infections, while aberrant Th2 responses have been associated with allergy and asthma. In experimental crescentic GN, some Th2-associated cytokines are reno-protective [9]. The signal transducer and activation of transcription (STAT) proteins provide a direct link between cytokine receptors and cytokine induced gene transcription [10]. Activation of the interleukin (IL)-4 receptor on undifferentiated T cells results in the activation of STAT6 with expression of IL-4 related genes [11].

STAT6 is considered central to mounting effective Th2 responses, including the production of Th2 cytokines IL-4 and IL-5, and the key transcription factor GATA binding protein 3 (GATA3) [12]. STAT6-deficient mice have

impaired Th2 immune responses, but otherwise are phenotypically normal and produce normal numbers of $CD4^+$ T cells [13]. While early studies suggested that STAT6 was an absolute requirement for IL-4 production [14,15], subsequently it was demonstrated that STAT6-deficient mice can produce IL-4 in response to parasitic infection [16,17]. STAT6 deficiency is protective in several Th2-associated disease models, including allergic asthma [18,19] and eosinophilia with airway hypersensitivity [20]. In experimental lupus nephritis, $STAT6^{-/-}$ mice were significantly protected from renal injury, despite high levels of lupus associated autoantibodies [21]. However, in other models, including sepsis [22] and kidney ischaemia reperfusion injury [23], organ inflammation and damage was enhanced in $STAT6^{-/-}$ mice.

We sought to define a role for STAT6 in the production of nephritogenic immunity and renal injury in experimental crescentic GN. We administered sheep anti-mouse GBM globulin to C57BL/6 wild-type (WT) and $STAT6^{-/-}$ mice (on a C57BL/6 background). Early immune responses demonstrated systemic up-regulation of the key Th1 and Th17 transcription factors, T-bet and Rorγ, respectively, in $STAT6^{-/-}$ mice on day 6. Autologous renal injury, assessed after 21 days, demonstrated enhanced histological and functional renal injury in $STAT6^{-/-}$ mice, with exaggerated nephritogenic Th1 and Th17 cellular immunity and decreased IL-5 production in $STAT6^{-/-}$ mice. The results demonstrate that STAT6 regulates Th1 and Th17 immune responses and attenuates experimental crescentic GN.

Materials and methods

Experimental design and statistics

STAT6-deficient ($STAT6^{-/-}$) mice on a C57BL/6J background were obtained from the Jackson Laboratories (Bar Harbor, ME, USA) and bred at Monash Medical Centre (Melbourne, Australia). C57BL/6J WT mice were obtained from Monash Animal Services (Melbourne, Australia). Sheep anti-mouse GBM antibody was generated as described previously [24]. Autologous phase anti-GBM GN was induced in age-matched, 8- to 10-week-old male mice after intravenous (i.v.) injection of 15 mg of sheep anti-mouse GBM antibody (day 0). Immune responses and/or renal injury were measured on days 6 and 21. In the experiments performed on day 6, four mice were used to assess transcription factor expression and seven mice to assess cytokine number and production. In day 21 experiments six to seven mice were used in each group; experiments were performed twice to ensure validation of the results. Studies were performed in accordance with National Health and Medical Research Council of Australia guidelines and approved by the Monash University Animal Ethics Committee. Results are expressed as mean ± standard error of the mean (s.e.m.). For statistical analysis, unpaired *t*-test was used (GraphPad Prism; GraphPad Software, San Diego, CA, USA). A value of $P < 0{\cdot}05$ was considered statistically significant.

Assessment of renal injury

Glomerular abnormalities were assessed on periodic acid Schiff (PAS)-stained, Bouin's fixed, 3-μm-thick, paraffin-embedded sections using coded slides. Glomerular crescent formation was defined as two or more layers of cells in Bowman's space (in ≥50 glomeruli per mouse). Semi-quantitative analysis of tubulointerstitial damage was performed on these sections, using a protocol described previously [7]. From each animal 10 randomly selected cortical medium power fields were examined. Injury was defined as tubular dilatation, tubular atrophy, sloughing of tubular epithelial cells or thickening of the basement membrane. Each cortical field was allocated a score according to the amount of injury observed within the tubulointerstitial field (0, no tubulointerstitial damage; 1, less than 25%; 2, 25–50%; 3, 50–75%; 4, >75% of the tubulointerstitial field damaged). The percentage of abnormal glomeruli was determined by examining a minimum of 50 glomeruli/mouse for abnormalities according to previously published protocols [25]. Abnormalities included glomerular hypercellularity, crescent formation, fibrinoid necrosis, segmental proliferation, hyalinosis and capillary wall thickening.

Urine was collected using metabolic cages for 24 h prior to the end of experiments. Proteinuria was determined using a modified Bradford assay and expressed as mg/24 h [7]. Serum creatinine measurements were recorded after termination of the experiment using an alkaline picric acid method and an auto-analyser.

Urinary nitric oxide (NO) was measured as described previously, using a Griess assay [25]. Urine samples (collected from mice for a 24-h period before killing) were centrifuged at 2000 ***g*** for 10 min. A total of 50-μl aliquots of urine were added to 50 μl of Griess reagent (1·5% sulphanilamide/0·15% naphthyl ethylene diamine) in a 96-well microtitre plate. Samples were incubated for 10 min at room temperature and absorbance read at 540 nm. Urinary nitrite concentration was determined from standards of sodium nitrite of known concentrations. Samples were tested in duplicate and measured as micromolars (μM) per 24 h.

Renal leucocyte accumulation immunohistochemistry

Kidneys were fixed in periodate lysine paraformaldehyde for 4 h, washed with 20% sucrose solution, and then frozen in liquid nitrogen. Tissue sections were cut and a three-layered immunoperoxidase technique was used to stain for $CD4^+$ T cells and macrophages. The primary antibodies used were GK1·5 for $CD4^+$ T cells [anti-mouse CD4; American Type Culture Collection (ATCC), Manassas, VA, USA] and FA/11 for macrophages (anti-mouse CD68, provided by Dr G. Koch, Cambridge, UK). The secondary antibody used was

rabbit anti-rat biotin (BD Biosciences, San Jose, CA, USA). A minimum of 20 consecutively viewed glomeruli were assessed per animal. Results are expressed as cells per glomerular cross section (c/gcs) described previously [7].

Splenic transcription factor mRNA expression

For measurement of T-bet, GATA3 and RORγ by reverse transcription–polymerase chain reaction (RT–PCR), 500 ng of RNA was treated with 1 unit of amplification grade DNase I (Invitrogen, Melbourne, Australia), primed with random primers (Applied Biosystems, Foster City, CA, USA) and reverse-transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems). Gene-specific oligonucleotide primers designed using the Primer 3 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) were synthesized by Invitrogen, using a protocol described previously [7]. A Rotor Gene RG-3000 (Corbett Research, Mortlake, Australia) using Power SYBR green PCR master mix (Applied Biosystems) was used to perform RT–PCR. PCR products were confirmed using melt-curve analysis while mRNA expression was quantified using serial dilutions of an exogenous standard. Primer sequences used were as described previously [7]. T-bet, GATA3 and RORγ expression was standardized to 18S (housekeeping gene) before being expressed as a fold increase relative to WT mice with GN.

Antigen-stimulated splenocyte cytokine production and circulating antigen-specific antibody titres

Using an aseptic technique, spleens were removed and the total number of splenocytes determined using a haemocytometer, with viability determined by trypan blue exclusion. Single cell suspension of splenocytes (4×10^6 cells/ml) were cultured in RPMI-1640/10% foetal calf serum (FCS) with protein G-purified normal sheep immunoglobulin (Ig)G (10 μg/ml) at 37°C for 72 h. There was no difference in splenocyte numbers between WT and STAT6$^{-/-}$ mice on days 6 or 21. IFN-γ, IL-4 and IL-17A concentrations were measured by enzyme-linked immunosorbent assay (ELISA) as described previously [26]. The following antibodies were used: rat anti-mouse IFN-γ (R4-6A2; BD Pharmingen, San Diego, CA, USA), biotinylated rat anti-mouse IFN-γ (XMG1·2; BD Pharmingen), rat anti-mouse IL-4 (11B11; ATCC), biotinylated rat anti-mouse IL-4 (BVD6; DNAX, Palo Alto, CA, USA), anti-mouse IL-10 (BD Pharmingen 18141D) and biotinylated rat anti-mouse IL-10 (BD Pharmingen 18152D). For IL-17A concentrations an IL-17A DuoSet ELISA Kit (R&D Systems, Minneapolis, MN, USA) was used. For detection of IL-5 production, rat anti-mouse IL-5 (R&D Systems) and biotinylated rat anti-mouse IL-5 (R&D Systems) were used as described previously [27].

ELISA was used to detect circulating serum antigen-specific IgG titres [28] with serial dilutions of sera: 1:50–1:3200. For measurement of IgG1, IgG2b and IgG2c sera were tested at serial dilutions (1:50, 1:200 and 1:1000) using biotinylated rat anti-mouse antibodies (BD Pharmingen). Results are expressed as optical density $(OD)_{450} \pm$ s.e.m.

Results

STAT6 deficiency results in exaggerated histological and functional renal injury

To define the role of STAT6 on experimental crescentic GN, we administered sheep anti-mouse GBM globulin to WT and STAT6$^{-/-}$ mice. Experiments ended 21 days later when WT mice had developed diffuse proliferative and crescentic GN with moderate tubulointerstitial injury (Fig. 1a and b). Compared to the renal injury observed in WT mice, injury was enhanced in STAT6$^{-/-}$ mice with GN (Fig. 1c and d). The proportion of glomeruli which demonstrated crescent formation was increased in STAT6$^{-/-}$ mice compared to WT mice with GN (Fig. 1e). Assessing tubulointerstitial injury, using a semi-quantitative assessment of periodic acid-Schiff (PAS)-stained sections, injury and inflammation was increased significantly in STAT6$^{-/-}$ mice (Fig. 1f). Consistent with enhanced glomerular crescent formation glomerular leucocyte recruitment was increased in STAT6$^{-/-}$ mice. The number of macrophages (Fig. 1g) and CD4$^+$ T cells (Fig. 1h) observed per glomerulus was increased in STAT6$^{-/-}$ mice compared to WT mice with GN. On day 21, WT mice had developed characteristic hallmarks of functional renal injury with increased proteinuria and elevated serum creatinine. However, functional injury was enhanced in STAT6$^{-/-}$ mice, with increased urinary NO production, elevated proteinuria and a trend towards increase in serum creatinine (Fig. 2a–c).

Systemic nephritogenic immune responses demonstrate a more predominant Th1 and Th17 phenotype in STAT6$^{-/-}$ mice

The nature and direction of the systemic immune response influences the pattern and severity of glomerular disease, therefore we measured immune responses directed against the nephritogenic antigen (i.e. sheep globulin). On day 21 systemic Th1 and Th17 cellular immune responses, assessed by antigen-stimulated splenocyte cytokine production, were increased in STAT6$^{-/-}$ mice. Production of the key Th1-produced cytokine, IFN-γ, and key Th17-produced cytokine, IL-17A, were increased in STAT6$^{-/-}$ mice (Fig. 3a and b). In contrast, when assessing Th2 responses, there was no difference in IL-4 production (Fig. 3c); however, production of IL-5 was decreased significantly in STAT6$^{-/-}$ mice (Fig. 3d). In addition, we measured IL-10 production from splenocyte cultures; however, levels were below the detection level of the assay in WT and STAT6$^{-/-}$ mice. These results demonstrated

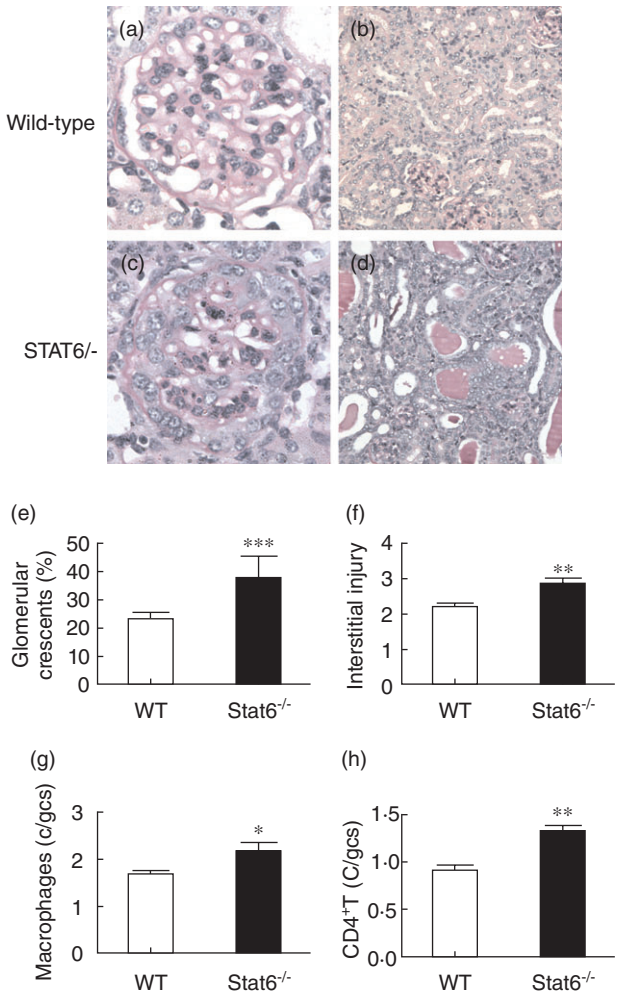

Fig. 1. Histological renal injury and accumulation of cellular effectors of injury in wild-type (WT) ($n = 7$) and signal transducer and activation of transcription 6 (STAT6$^{-/-}$) ($n = 6$) mice administered sheep anti-mouse glomerular basement membrane (GBM) globulin. Twenty-one days after the administration of sheep anti-mouse GBM globulin, WT mice developed crescentic glomerulonephritis (GN) with fibrinoid necrosis (a) (400× magnification) and tubulointerstitial injury (b) with hypercellularity, tubular dilatation and atrophy (200× magnification). Compared to the renal injury seen in WT mice, STAT6$^{-/-}$ mice demonstrated significantly worse glomerular (c) and tubulointerstitial injury (d). Representative photomicrographs are demonstrated. On day 21 STAT6$^{-/-}$ mice had more glomerular crescents (e) and tubulointerstitial injury (f) than WT mice with GN. Furthermore, increased numbers of macrophages (g) and CD4$^+$ T cells (h) were seen per glomerular cross section (gcs) in STAT6$^{-/-}$ mice. $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

heightened Th1 and Th17 systemic immunity with a partial attenuation in Th2 responses.

Humoral immune responses were assessed by measuring circulating antibody levels against the nephritogenic antigen. While WT mice with GN developed easily detectable antigen-specific humoral immune responses, there was a trend towards a decrease in measurable immunoglobulin (IgG) levels directed against the nephritogenic antigen in STAT6$^{-/-}$ mice (Fig. 4a). Assessing IgG subtype production demonstrated a statistically significant decrease in antigen-specific IgG1 in STAT6$^{-/-}$ mice at serial dilutions, while production of antigen-specific IgG2b and IgG2c was unchanged.

In STAT6$^{-/-}$ mice expression of the key Th1 and Th17 transcription factors is increased on day 6, while there is no difference in renal injury

While the key Th1 (T-bet) [7] and Th17 (Roryt) [8] transcription factors influence the severity of renal injury in experimental crescentic GN, expression of these transcription factors peaks early in the disease process [7]. We measured expression of the key transcription factors and cytokines after 6 days. No difference in splenic GATA3 expression was observed between WT and STAT6$^{-/-}$ mice. However, there was a significant increase in T-bet and Rorγ expression in STAT6$^{-/-}$ mice compared to WT mice given sheep anti-mouse GBM serum (Fig. 5a–c).

There was no difference in splenocyte numbers in WT and STAT6$^{-/-}$ mice injected with sheep anti-mouse GBM serum (Fig. 6a). Antigen-stimulated cytokine production demonstrated a trend towards increased production of IFN-γ and IL-17A in STAT6$^{-/-}$ mice (Fig. 6b and c). While production of IL-4 was detected readily in all samples, no difference was observed between WT and STAT6$^{-/-}$ mice (Fig. 6d). However, IL-5 production was decreased significantly in STAT6$^{-/-}$ mice on day 6 (Fig. 6e). There was no difference in antibody levels between WT and STAT6$^{-/-}$ mice on day 6; levels were not elevated compared to untreated mice. We analysed renal injury in WT and STAT6$^{-/-}$ mice 6 days after the administration of sheep anti-mouse GBM globulin. No difference was seen in histological renal injury (WT 36·9 ± 4·7 *versus* STAT6$^{-/-}$ 37·6 ± 3·1% abnormal glomeruli) urinary proteinuria (WT 6·1 ± 1·1 *versus* STAT6$^{-/-}$ 7·4 ± 1·8 mg/24 h) or serum creatinine (WT 20·7 ± 0·8 *versus* STAT6$^{-/-}$ 18·0 ± 1·1% μmol/l).

Discussion

Experimental crescentic GN was enhanced significantly in the absence of endogenous STAT6. We found that STAT6-deficient mice demonstrated more glomerular crescents and tubular interstitial injury as well as increased proteinuria and urinary nitrate production with a trend towards increased serum creatinine. These data demonstrated a protective role for STAT6 in experimental crescentic GN. While STAT6$^{-/-}$ mice developed attenuated injury in some models of Th2-driven disease [18–20], both injurious [21] and protective roles [23] have been described in experimental renal disease. In addition to demonstrating a renal protective role for STAT6 in crescentic GN, we found enhanced nephritogenic immunity; including increased IFN-γ and IL-17A production in STAT6$^{-/-}$ mice on day 21.

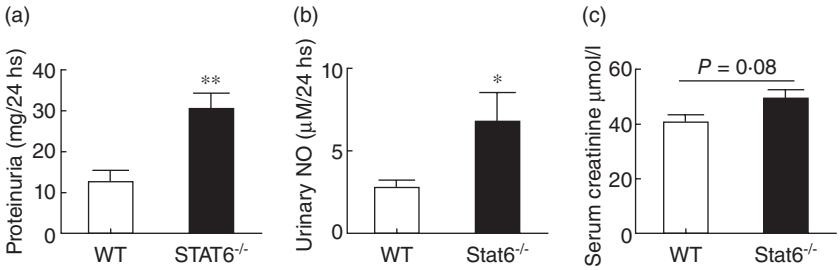

Fig. 2. Functional markers of renal injury 21 days after administration of sheep anti-mouse glomerular basement membrane (GBM) globulin. Compared to wild-type (WT) mice ($n = 7$) with glomerulonephritis (GN), functional renal injury assessed by urinary protein excretion (a) and urinary nitric oxide (NO) (b) was increased in signal transducer and activation of transcription 6 (STAT6$^{-/-}$) mice ($n = 6$) with GN. Consistent with these observations, there was a trend towards increased serum creatinine levels in STAT6$^{-/-}$ mice compared to WT controls (c). *$P < 0{\cdot}05$; **$P < 0{\cdot}01$.

In planted antigen models of crescentic GN, CD4$^+$ T cells initiate the nephritogenic immune response [29] and act as important effector cells in disease [1,4]. The key Th1 transcription factor, T-bet [7], and pivotal cytokines IL-12 [30], IL-18 [26] and IFN-γ [24], mediate severe disease and mice deficient in these cytokines are afforded significant protection from disease. More recently we have demonstrated direct injurious roles for both Th1 and Th17 cells in a planted antigen model of GN [25]. Separately, we have shown that Rorγt mediates severe crescentic injury, independent of Th1 responses, in this model [8], while others have shown that deficiencies in Th17-associated cytokines afford significant protection [31]. In these experiments we found that the heightened Th1 and Th17 nephritogenic immune responses seen in STAT6$^{-/-}$ mice facilitated enhanced renal disease seen on day 21. Therefore, we concluded that endogenous STAT6 limits nephritogenic Th1 and Th17 immunity in crescentic GN. In parallel with the enhanced nephritogenic immunity seen in STAT6$^{-/-}$ mice, we found decreased production of selected Th2-associated cytokines and Th2-associated antibody subtypes (IgG1).

The role of Th2 cells and their associated cytokines in experimental crescentic GN is less clearly defined. However, endogenous Th2-associated cytokines, IL-4 [32] and IL-10 [33], limit glomerular disease, while administration of IL-4 and/or IL-10 also lessens glomerular injury [28]. We found no difference in IL-4 or IL-10 production in STAT6$^{-/-}$ mice although production of IL-5, a key Th2 disease-modifying cytokine, was decreased. Enhanced IL-5 production has been associated with increased severity in Th2-mediated renal diseases [34]; however, it is plausible that IL-5 is protective in this model. Protection from allergic asthma in STAT6$^{-/-}$ mice seems to be largely IL-5-dependent. In a model of asthma and airway hyperresponsiveness, STAT6$^{-/-}$ mice were protected; however, when recombinant IL-5 was administered, protection was cancelled [20]. Similar to STAT6$^{-/-}$ mice, IL-5-deficient mice are protected from allergic asthma [35], while monoclonal anti-IL-5 therapy attenuates airway disease suc-

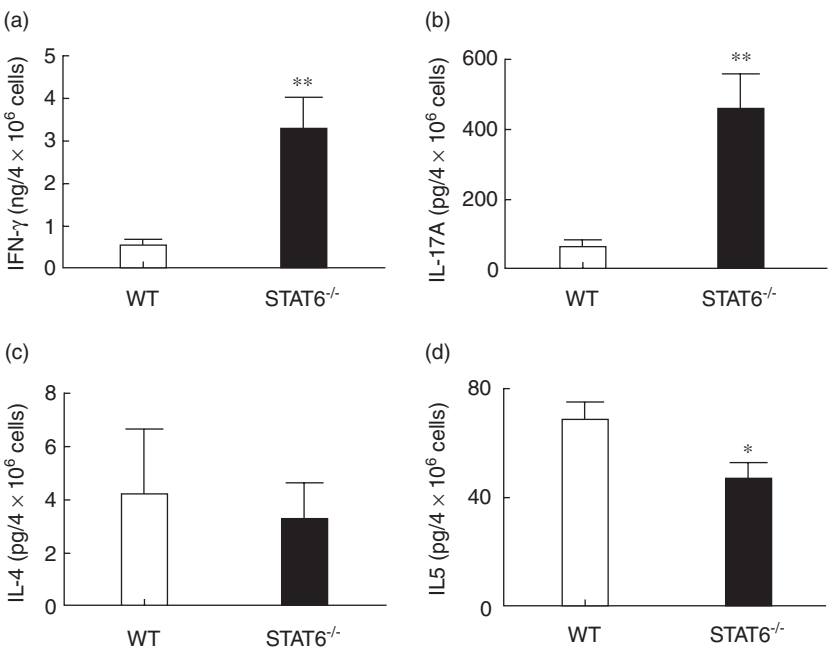

Fig. 3. Systemic immune responses to the nephritogenic antigen measured in wild-type (WT) ($n = 7$) and signal transducer and activation of transcription 6 (STAT6$^{-/-}$) ($n = 6$) mice, 21days after the administration of sheep anti-mouse glomerular basement membrane (GBM) globulin. On day 21 antigen-stimulated production of interferon (IFN)-γ (a) and interleukin (IL)-17A (b) were increased in STAT6$^{-/-}$ mice compared to WT mice. While there was no difference in interleukin (IL)-4 (c) production between the groups, IL-5 (d) was decreased significantly in STAT6$^{-/-}$ mice compared to WT mice with glomerulonephritis (GN). *$P < 0{\cdot}05$; **$P < 0{\cdot}01$.

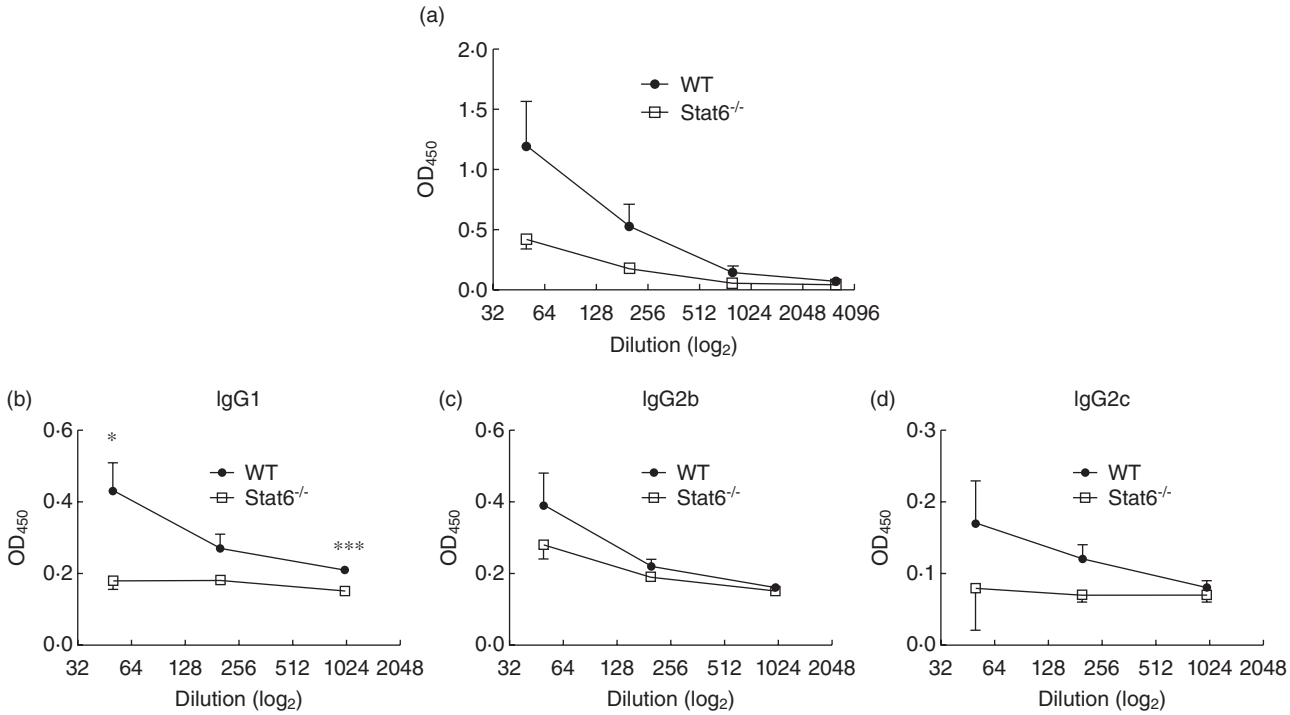

Fig. 4. Antigen-specific serum antibody titres measured in wild-type (WT) ($n = 7$) and signal transducer and activation of transcription 6 (STAT6$^{-/-}$) ($n = 6$) mice 21days after the administration of sheep anti-mouse glomerular basement membrane (GBM) globulin. Serum antigen-specific immunoglobulin (Ig)G (a) showed a trend towards decrease detection in STAT6$^{-/-}$ mice compared to WT mice. Subclass titres of IgG1 (b) were decreased in STAT6$^{-/-}$ mice at high and low dilutions. There were no detectable differences in serum IgG2b (c) and IgG2c (d) titres in WT and STAT6$^{-/-}$ mice. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

cessfully [36]. Therefore, it is likely that in crescentic GN, STAT6 activation results in IL-5 production which attenuates renal injury, possibly through the inhibition of Th1 and Th17 responses.

Assessing renal injury early in the disease process at day 6 demonstrated no difference between WT and STAT6$^{-/-}$ mice. These results confirmed that the injury seen on day 21 was a result of the heightened systemic immunity which developed between days 6 and 21, and not a reflection of an existing predisposition to renal injury in STAT6$^{-/-}$ mice. Interestingly, mRNA expression of both T-bet and Rorγt was increased in STAT6$^{-/-}$ mice, with a trend towards increased production of IFN-γ and IL-I7A on day 6. On day 21 differences in production of these cytokines by WT and STAT6$^{-/-}$ mice had reached statistical significance. Previous studies in STAT6$^{-/-}$ mice in experimental lymphoproliferative disease demonstrated that STAT6 deficiency resulted in a shift from a predominant Th2 phenotype towards production of Th1-associated cytokines. In these experiments no difference was observed in the production of Th17-associated cytokines [37]. Consistent with these results, Th1 differentiation occurred without the provision of extrinsic IFN-γ or IL-12 in conditional GATA3-deficient mice [38]. The ability of other key regulators to influence the associated and reciprocal Th cell lineages is well described.

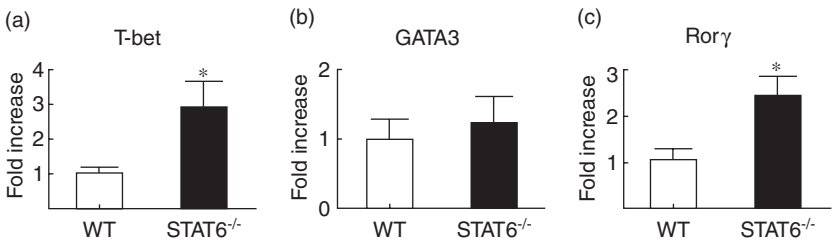

Fig. 5. Splenic mRNA expression of key T helper type 1 (Th1) (a), Th2 (b) and Th17 (c) transcription factors 6 days after the administration of sheep anti-mouse glomerular basement membrane (GBM) globulin. Systemic expression of T-bet and retinoic acid-related orphan receptor-γ (Rorγ) was increased in signal transducer and activation of transcription 6 (STAT6$^{-/-}$) ($n = 4$) compared to wild-type (WT) mice ($n = 4$) at this early time point. There was no difference in splenic GATA3 expression on day 6 between the different groups. $^{*}P < 0{\cdot}05$.

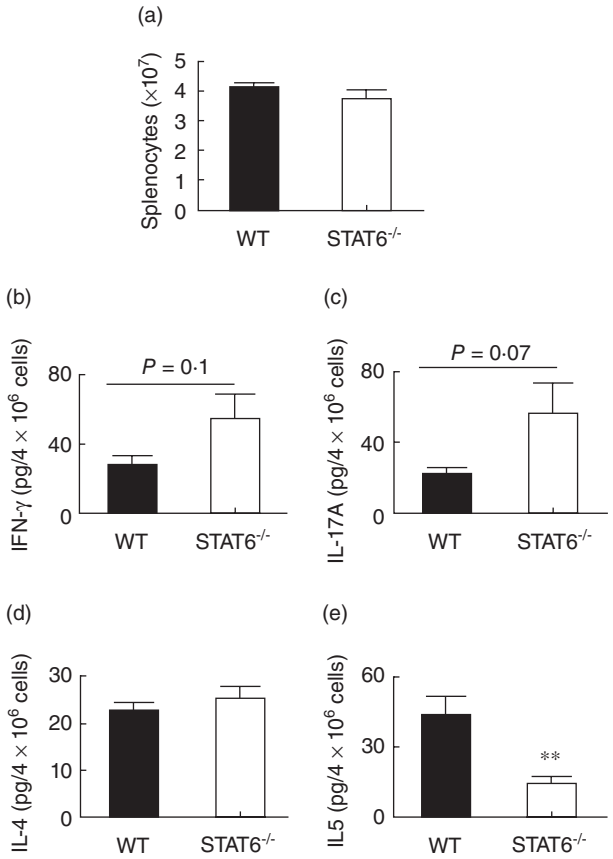

Fig. 6. Systemic immune responses to the nephritogenic antigen measured in wild-type (WT) and signal transducer and activation of transcription 6 (STAT6$^{-/-}$) mice 6 days after the administration of sheep anti-mouse GBM globulin. No difference in splenocyte numbers (a) was detected between WT ($n = 7$) and STAT6$^{-/-}$ mice ($n = 7$). While STAT6$^{-/-}$ mice demonstrated a trend towards increased interferon (IFN)-γ (b) and interleukin (IL)-17A (c) production, no difference in IL-4 (d) production was detectable between the groups. Production of IL-5 (e) was decreased significantly in STAT6$^{-/-}$ mice compared to WT mice with glomerulonephritis (GN). $^{**}P < 0{\cdot}01$.

T-bet, the key regulator of Th1 responses, can influence the Th17 phenotype. In experimental allergic encephalomyelitis, inhibition of T-bet by small interfering RNA inhibited the production of both Th1 and Th17 pathogenic responses [39]. Conversely, it has been suggested that T-bet negatively regulates the production of Th17 associated cytokines *in vitro* [40]; this was demonstrated *in vivo* in experimental Chagas' disease [41]. Taken together, these reports demonstrate that key Th1 transcription factors can influence the production of Th17 responses. We propose that STAT6 influences pathogenic Th1 and Th17 inflammatory responses in experimental crescentic GN. This novel finding suggests a greater role for Th2 cells in experimental crescentic GN than was previously appreciated. In addition to IL-4 and IL-10, it would seem that STAT6 with IL-5 production is required for control of nephritogenic immunity. Production of the regulatory Th2-related cytokines is required not only for regulation of inflammatory Th1 responses but also for regulation of Th17 systemic immunity.

In conclusion, we found that STAT6$^{-/-}$ mice developed increased expression of key Th1 and Th17 transcription factors early in the disease. This resulted in increased Th1 and Th17 nephritogenic immunity on day 21. Production of a key Th2-related cytokine, IL-5, was decreased consistently during the disease state. Enhanced nephritogenic immune responses resulted in enhanced renal injury in STAT6$^{-/-}$ mice.

Acknowledgements

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Disclosure

None.

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