Short Communication

Transferred Antigen-Specific T_H17 but not T_H1 Cells Induce Crescentic Glomerulonephritis in Mice

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To explore the role of antigen-specific CD4⁺ T cells in glomerulonephritis, we administered ovalbumin 323–339 peptide conjugated to glomerular-binding polyclonal antibody and induced disease in RAG1^{-/-} mice with CD4⁺ T cells from OT2 × RAG1^{-/-} mice. These OT2 × RAG1^{-/-} mice have a transgenic T-cell receptor specific for this peptide. When CD4⁺ T cells were primed *in vivo*, crescentic glomerulonephritis developed after 21 days in mice given peptide-conjugated glomerular-binding antibody but not unconjugated antibody control. We then investigated the relative roles of T_H1 and T_H17 cells, using Fab₂ fragments of glomerular-binding antibody to exclude a role for antibody in this model. T cells from OT2 × RAG1^{-/-} mice were polarized *in vitro*, and T_H1 or T_H17 cell lines were injected into mice that were also given peptide-conjugated Fab₂ or unconjugated Fab₂ control, giving four experimental groups. After 21 days crescentic glomerulonephritis was seen in mice receiving T_H17 cells and peptide-conjugated Fab₂ but in none of the other three groups. These results suggest that T_H17 but not T_H1 cells can induce crescentic glomerulonephritis. (*Am J Pathol* 2011, 179:2683–2690; DOI: 10.1016/j.ajpath.2011.08.017)

The ability of passively administered antibody to cause glomerulonephritis has been long established,¹ and studies in Fc receptor–deficient mice have confirmed a role for immune complexes in both autologous nephrotoxic nephritis and lupus models.^{2,3} Evidence for T-cell-mediated, antibody-independent disease was suggested in early studies of lymphocyte transfer⁴ and more recently in murine autologous nephrotoxic nephritis using B-cell–deficient mice.⁵ T-cell effector mechanisms have also been demonstrated in a rat model of anti–glomerular basement membrane (GBM)

disease⁶ and in a mouse model of antineutrophil cytoplasmic antibody–associated vasculitis.^{7,8} The subset of T_H17 CD4⁺ T cells has recently emerged as a key mediator of tissue damage in a number of systems where T_H1 cells were previously thought to play a prominent role.⁸ Their pathogenicity has been proven in colitis, multiple sclerosis, and arthritis models.^{9–12} However, the suggestion that T_H17 cells are pathogenic in all cases and T_H1 cells are not is likely to be simplistic,¹³ and data have shown that both T_H1 and T_H17 cells can induce disease in uveitis and multiple sclerosis.^{14,15}

Work based largely on studies in the autologous nephrotoxic nephritis model has suggested that T_H1 CD4⁺ effector mechanisms are central in crescentic glomerulonephritis,¹⁶ although importantly studies in IL-12–deficient mice were performed in mice deficient for the p40 subunit shared by IL-12 and IL-23.¹⁷ Therefore, the relative ability of T_H17 and T_H1 effector CD4⁺ T cells to induce glomerulonephritis was not clear. Further data have suggested that IL-17 is a key mediator in this model,¹⁸ although several cell types produce IL-17 and direct evidence of a role for T_H17 cells was not presented in this study. A recent report addressed the role of T_H17 cells using mice with a transgenic T-cell receptor specific for ovalbumin and a model in which ovalbumin was planted on the GBM.¹⁹ In this article, both T_H1 and T_H17 cells were shown to induce glomerulonephritis, but only T_H1 cells caused crescent formation. We have used a similar experimental system and have found that only T_H17 cells induce glomerulonephritis with crescent formation and that T_H1 cells do not induce significant disease.

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Materials and Methods

Mice

OT2 mice²⁰ on a C57BL/6 background were obtained from Charles River (Paris, France) and then bred in house. RAG1^{-/-} mice²¹ also on a C57BL/6 background were obtained from the Jackson Laboratory (Bar Harbor, ME) and bred in house. OT2 and RAG1^{-/-} mice were interbred to obtain OT2 × RAG1^{-/-} mice with mice genotyped by PCR. Mice were bred under specified pathogen-free conditions, and all experiments were performed according to local and UK home office regulations.

Preparation and Conjugation of Glomerular-Binding Antibody

Polyclonal glomerular-binding serum was raised in sheep as previously described.²² For initial experiments, this serum was purified with protein G chromatography. A conjugation strategy was developed in which sulfhydryl groups from cysteine on the peptide would react with maleimide groups on the antibody. Modified ovalbumin 323–339 was commercially synthesized (Cambridge Research Biochemicals, Billingham, UK) as a 19-amino acid peptide with an additional cysteine residue at the N terminus and an additional biotinylated lysine added to the C terminus. The addition of biotin was to allow characterization of the final product. Antibody was maleimide activated by reaction with sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate. After buffer exchange by gel filtration, the peptide was reacted with the maleimide-activated antibody. The product was dialyzed into PBS. The final product was then characterized. The molar biotin:IgG ratio of the product was assessed because each peptide contained one biotin. 4'-Hydroxyazobenzene-2-carboxylic acid undergoes a color change with biotin, and using a standard curve of known biotin concentrations, we calculated the biotin:IgG ratio of our conjugated antibody. In later experiments, we prepared Fab₂ fragments of the glomerular-binding antibody. This was performed based on a previously published method.²³ Digested fragments were removed using a centrifugal device with a 30-kDa membrane (Vivaspin 20). Repeated concentration and dilution in 150 mmol/L NaCl and 20 mmol/L piperazine (pH 6) were performed. The Fab₂ preparation was then passed through Hitrap Q and protein G columns (GE Healthcare, Little Chalfont, UK) to remove any residual pepsin or whole antibody, respectively. The run through was then concentrated (Vivaspin 20) and buffer exchanged into PBS with 1 mmol/L EDTA for conjugation to modified ovalbumin 323–339 as described for whole antibody. At the end of the conjugation, the product was buffer exchanged to 150 mmol/L NaCl and 20 mmol/L piperazine (pH 6) using gel filtration. SDS-PAGE confirmed that there was no detectable whole antibody in the preparation (Figure 1D).

Induction and Assessment of Disease

RAG1^{-/-} mice were injected with 10⁶ splenocytes from OT2 × RAG1^{-/-} mice. Seven days later, they were injected with 0.132 mg/g of peptide-conjugated glomerular-binding antibody or unconjugated antibody. They were sacrificed 21 days later, with the last 24 hours spent in metabolic cages for urine collection. At the end of the experiment, spleens were cultured in complete medium [Dulbecco's modified Eagle's medium; HEPES, 15 mmol/L; 2-beta-mercaptoethanol, 50 μmol/L; and fetal calf serum, 10% (Invitrogen, Paisley, UK)] for 48 hours, and cytokines were assessed by enzyme-linked immunosorbent assay (ELISA) in supernatants. ELISA kits were from R&D Systems (Abingdon, UK). Disease was assessed by measuring albuminuria with radial immunodiffusion and serum creatinine with electrospray mass spectrometry as previously described.^{22,24} Kidneys were fixed in Bouin's fixative and stained with PAS for assessment of histologic features. Neutrophils were identified by their characteristic nuclear morphologic findings. Samples were also fixed in phosphate-lysine-periodate and then frozen for immunofluorescence staining. CD4 (clone L3T4; BD Biosciences, Oxford, UK) and CD68 (clone FA11; AbD Serotec, Oxford, UK) staining was detected using fluorescein isothiocyanate (FITC) mouse anti-rat IgG (Jackson ImmunoResearch Laboratories Inc, West Grove, PA) followed by Alexa fluor 488 goat anti-FITC (Invitrogen). For quantitation of glomerular cells, at least 40 glomerular cross-sections (GCSs) were assessed, and for interstitial cells, at least five randomly selected fields at ×400 magnification were assessed.

T-Cell Lines

Single-cell suspensions were prepared from spleens of OT2 × RAG1^{-/-} mice in complete medium (as above). Cells were cultured with ovalbumin 323–339 peptide at 2 μg/mL at 5 × 10⁶/mL in polarizing conditions as follows. For T_H1 development, 10 ng/mL of recombinant murine IL-12 (BD Biosciences) and 5 μg/mL of neutralizing anti-IL-4 antibody (11B11; BD Biosciences) were used. For T_H17 development, 25 ng/mL of recombinant murine IL-6 (Peprotech EC, London, UK), 3 ng/mL of human transforming growth factor-β (R&D), 10 ng/mL of recombinant murine IL-23 (R&D), and 10 μg/mL of anti-interferon (IFN)-γ (XMG1.2; BD Biosciences) were used. At day 3, 10 ng/mL of IL-2 and IL-23 were added for T_H1 and T_H17 culture, respectively. For the day 21 experiment, at day 6 cells were restimulated with irradiated splenocytes at a ratio of 5:1 in fresh medium and polarizing conditions. Cells were harvested after 3 days (total of 9 days in culture). For the day 14 experiment, cells were used after 7 days without restimulation. In this experiment, cells from OT2 rather than OT2 × RAG1^{-/-} mice were used because of the availability of the former. All cells used *in vivo* were analyzed as described under intracellular cytokine staining. To induce glomerulonephritis with the T-cell lines, RAG1^{-/-} mice were injected i.v. with 5 × 10⁶ cells per mouse. Immediately after this, they were given 0.25 mg/g of ovalbumin peptide-conjugated Fab₂ glomerular-binding

Figure 1. **A:** Coomassie-stained SDS-PAGE (reduced) of glomerular-binding IgG before and after conjugation with peptide (three dilutions of each). The heavy chain of the conjugate has a higher molecular weight, and three bands are visible for the light chain, each corresponding to a light chain with differing numbers of peptide molecule added (arrows). **B:** Immunofluorescence staining for sheep IgG was performed on kidney from mice injected with conjugated or unmodified IgG, and the intensities were similar. **C:** CD4⁺ T cells from OT2 $\times$ RAG1^{-/-} mice proliferated *in vitro* when cultured with OT2 peptide and peptide-conjugated IgG, but not with IgG alone. Data are from a ³H thymidine proliferation assay using 2×10^5 spleen cells/mL with 2 mg/mL peptide and conjugated IgG containing equimolar amounts of peptide. **D:** Coomassie-stained SDS-PAGE (nonreduced) of glomerular-binding IgG before and after digestion to yield Fab₂ fragments. There was no detectable intact IgG.

antibody or unconjugated Fab₂ kidney binding antibody. They were sacrificed up to 21 days later, with the last 24 hours spent in metabolic cages for urine collection. At the end of the experiment, single cell suspensions were prepared from spleens. For each mouse in the day 21 experiment, one-third of each kidney was taken and digested using a previously published method.²⁵ After digestion, cells were further purified using a Ficoll separation.

Intracellular Cytokine Staining

Restimulation and intracellular staining was performed as previously described except that Brefeldin rather than Monesin was used.²⁶ The same method was used for analysis of the polarized T-cell lines or spleen and kidney cells at the end of the experiments using these T-cell lines. Antibodies used for flow cytometry were from BD Biosciences as follows: phycoerythrin (PE) and IL-17 (TC11-18H10), FITC and IFN- γ (XMG1.2), and PECy5 and CD4 (H129.19). Analysis was performed on a Cyan (Dako Cytomation, Ely, UK) or a BD FACScalibur flow cytometer (BD Biosciences).

Statistical Analyses

Statistical analyses were performed using GraphPad Prism Software (GraphPad Software Inc, San Diego, CA). An unpaired Student's *t*-test was used to compare two groups. If more than two groups were compared, a one-way analysis of variance with Tukey's posttest was used. If the variances were significantly different, a logarithmic transformation was performed before analysis.

Results

Characterization of Conjugated Antibody and Fab₂ Fragments

We conjugated the ovalbumin 323–339 peptide recognized by OT2 T cells to glomerular-binding sheep IgG to target this to the glomerulus (Figure 1). The heavy chain of the conjugate has a higher molecular weight, and three bands are visible for the light chain, corresponding to different numbers of peptide molecules added (Figure 1A). When conjugated and unconjugated antibody was injected intravenously, binding was similar on immunofluorescence staining kidney section (Figure 1B). We confirmed that peptide from the conjugate could be presented and stimulated OT2 cells *in vitro* (Figure 1C). Fab₂ fragments were generated by digestion, and we confirmed that they did not contain whole IgG (Figure 1D). The conjugates were assayed for biotin, and the biotin: IgG ratio (and hence peptide: IgG ratio) of our conjugated whole IgG antibody was found to be 4.7. For the Fab₂ conjugate, there were 3.25 biotins and thus 3.25 peptides per Fab₂ molecule. We also confirmed that peptide-conjugated Fab₂ bound to the glomerular capillary wall when injected *in vivo* (not shown).

Figure 2. Disease was induced in RAG1^{-/-} mice reconstituted with OT2 × RAG1^{-/-} spleen cells as described in *Materials and Methods*. **A–F:** In mice given peptide-conjugated glomerular-binding IgG there was albuminuria and crescent formation, with glomerular and interstitial CD68⁺ and CD4⁺ cell infiltrates, which were significantly greater than in mice given an equal amount of unconjugated glomerular-binding IgG ($P < 0.001$ for all parameters). **G:** Representative histologic analysis on PAS-stained sections from the experiment shown in **A–F**, with immunofluorescence staining for CD68⁺ macrophages and CD4⁺ cells. The glomeruli are darker than the surrounding autofluorescent tubules. **H:** Splenocytes were restimulated with peptide, and there was production of IFN-γ, IL-4, and IL-17 in cells from mice given peptide-IgG, which was not seen in mice given IgG alone. For each mouse, spleen cells were also cultured without peptide, and cytokine levels were below the detection of the ELISA in all cases. Each symbol in **A–H** represents data from an individual mouse.

Antigen-Specific CD4⁺ T Cells Induce Glomerulonephritis

In initial experiments, we aimed to show that antigen-specific OT2 T cells could induce glomerulonephritis using this approach. We transferred spleen cells from OT2 × RAG1^{-/-} mice into RAG1^{-/-} mice and 7 days later injected them with glomerular-binding IgG that had been conjugated with OT2 peptide or with unconjugated glomerular-binding IgG as a control. These mice had no CD8⁺ cells or B cells, and all CD4⁺ T cells had a receptor specific for the ovalbumin 323–339 peptide. After 21 days there was significant albuminuria in mice given peptide-conjugated antibody but not unconjugated antibody as shown in Figure 2A. No significant difference was found in serum creatinine concentrations between groups with levels of less than 15 μmol/L in all mice. Histologic parameters also showed disease in mice given peptide-conjugated antibody but not control (Figure 2, B–F), with increased crescent formation, glomerular CD4⁺ T cells, glomerular CD68⁺ macrophages, interstitial CD4⁺ T cells, and interstitial CD68⁺ macrophages. There were few glomerular neutrophils seen, and these were less than 0.08 per GCS in all mice. Representative light microscopy and immunofluorescence staining for CD4 and CD68 is shown in Figure 2G. Splenocytes from experimental animals were restimulated with ovalbumin 323–339 peptide, and production of IL-17, IFN-γ, and IL-4 was greater in mice given peptide-conjugated antibody than in those given unconjugated antibody control,

suggesting that an immune response, including T_H1, T_H2, and T_H17 cells, had been generated and that any of these cells could have caused glomerular injury (Figure 2H). In these experiments, unconjugated antibody did not induce significant disease, and the disease seen in mice given ovalbumin peptide-conjugated antibody was therefore caused by the OT2 × RAG1^{-/-} CD4⁺ T cells that recognized this peptide.

T_H17 but not T_H1 Cells Induce Crescentic Glomerulonephritis

To explore the relative roles of T_H1 and T_H17 cells in this model, we prepared polarized CD4⁺ T-cell lines *in vitro*, using T cells from OT2 × RAG1^{-/-} mice. In the initial experiments, we could not exclude the possibility that disease was partially dependent on the glomerular-binding antibody used to target the peptide to the glomerulus. As a further refinement to our experimental system, we therefore prepared Fab₂ fragments of glomerular-binding antibody and used these to target ovalbumin peptide to the glomerulus (Figure 1D). This system therefore totally excluded an antibody-mediated component, and glomerulonephritis was entirely mediated by OT2 CD4⁺ T cells. T_H1 or T_H17 cells were injected intravenously, immediately followed by an injection of peptide-conjugated Fab₂ fragments or unconjugated Fab₂ fragments as a control, giving four experimental groups. Three independent cell lines of T_H1 or T_H17 polarity were generated. Any given

Figure 3. A: OT2 $\times$ RAG1^{-/-} splenocytes were cultured in polarizing conditions and FACS staining showed that CD4⁺ cells of the desired polarity were obtained using these methods. The plots shown are from three independently cultured cell lines (derived from different mice) of each type that were used in this experiment. B–F: Polarized T_H1 or T_H17 CD4⁺ T cells were given to RAG1^{-/-} mice followed by an injection of peptide-conjugated Fab₂ nephrotoxic antibody or unconjugated Fab₂ antibody. Each mouse in each group was given a different cell line and so is an independent data point, and each symbol is an individual mouse. The data show that at day 21 glomerular CD68⁺ macrophage and CD4⁺ T-cell infiltration with crescent formation developed only in mice given peptide-conjugated antibody and T_H17 cells but not in mice given T_H17 or T_H1 cells with unconjugated Fab₂ or in mice given T_H1 cell with peptide-conjugated Fab₂ ($P < 0.001$ for all comparisons of glomerular histologic analysis). The interstitial CD4⁺ cell infiltrate was also increased in this group ($P < 0.01$). The interstitial macrophage infiltrate was greater after T_H17 cells in both the presence and absence of peptide (but not significant when all four groups were compared). G: Representative histologic analysis on PAS-stained sections from the experiment shown in B–F, with immunofluorescence staining for CD68⁺ macrophages and CD4⁺ cells. The glomeruli are darker than the surrounding autofluorescent tubules. H: Intracellular cytokine staining to examine the polarity of CD4⁺ T cells in mice that had been given T_H1 or T_H17 cells and peptide-conjugated Fab₂. Each bar is a separate mouse from the experiment depicted. We also digested kidneys from mice that had been given T_H17 cells and peptide-conjugated Fab₂ and performed intracellular cytokine staining on isolated CD4⁺ T cells, which showed that they had retained their polarity. No data are shown for the other groups because kidneys did not contain significant T-cell numbers (B and E). I: Representative flow cytometry plots gated on CD4⁺ cells. Although the percentages are lower than those seen in the original cell lines (A), this probably represents technical issues around isolating splenic and renal T cells and performing intracellular cytokine staining, and the data clearly show that polarity had been retained.

donor OT2 $\times$ RAG1^{-/-} mouse contributed cells to one T_H1 and one T_H17 culture only, so that no two T_H1 or T_H17 cultures derived from the same donors, and we had three independent T_H1 and T_H17 cultures, derived from three groups of donors. Each of these six cell lines were given

to one mouse that received peptide-conjugated Fab₂ and to one mouse that received unconjugated Fab₂ control. The data from this experiment are shown in Figure 3. Figure 3A shows that T_H1 and T_H17 cells of the desired polarity were obtained for all six cell lines used. A

Figure 4. A further experiment was performed with two T_H1 and two T_H17 OT2 cell lines (T_H1A , T_H1B , T_H17A , T_H17B) that were generated and injected into $RAG1^{-/-}$ mice followed by an injection of peptide-conjugated Fab_2 nephrotoxic antibody or unconjugated Fab_2 antibody. **A:** The polarity obtained *in vitro* for each cell line before injection is shown. Cell lines denoted A or B were derived from different donor OT2 mice, and each recipient mouse received one of these four cell lines. **B–F:** Histologic data at day 14 after induction of disease. Significantly more glomerular macrophages (**B**) and CD4 T cells (**C**) were found in mice given T_H17 cells and peptide-conjugated Fab_2 compared with other groups ($P < 0.001$ for all comparisons). **D:** There were occasional crescents seen but only in the T_H17 group ($P < 0.001$ compared with other groups). **E:** There were more interstitial macrophages in mice given T_H17 cells ($P < 0.05$ compared with both T_H1 groups and $P < 0.01$ compared with the T_H17 control group). **F:** There were also more interstitial CD4 T cells in mice given T_H17 cells ($P < 0.001$ compared with other groups). **G:** There were no differences in absolute numbers of CD4 $^{+}$ cells in spleens taken from mice at day 14. **H:** Intracellular cytokine staining from spleen cells for IL-17 and IFN- γ showed that T_H1 and T_H17 cells retained their polarity with data grouped according to the cell line received. **B–H:** Each symbol represents data from an individual mouse.

significant increase in glomerular CD4 $^{+}$ T cells and CD68 $^{+}$ macrophages, crescent formation, and increased interstitial CD4 $^{+}$ T cells were seen in mice given T_H17 cells and peptide conjugated Fab_2 but in none of the other groups (Figure 3, B–E). Crescent formation was also only seen in this group, and interstitial CD4 $^{+}$ T cells were increased. There was a trend toward more macrophages in both groups given T_H17 cells, but this did not reach statistical significance. We assessed glomerular neutrophil numbers, and these numbers were small with no differences between groups. For mice given T_H1 cells with conjugated Fab_2 , T_H1 cells with unconjugated Fab_2 , T_H17 cells with conjugated Fab_2 , and T_H17 cells with unconjugated Fab_2 , the numbers (mean $\pm$ SEM as cells per GCS) were 0.17 ± 0.15 , 0.06 ± 0.06 , 0.24 ± 0.19 , and 0.11 ± 0.08 , respectively. Variable proteinuria was found, but this was not associated with the histologic changes and was not greater in mice given peptide-conjugated Fab_2 compared with unconjugated Fab_2 . Albuminuria levels (mean $\pm$ SEM) for T_H1 , T_H1 control, T_H17 , and T_H17 control groups were 87 ± 23 , 77 ± 19 , 100 ± 75 , and 356 ± 176 μ g/d, respectively. In addition,

no significant difference was found in serum creatinine concentrations between groups with levels of less than 11 μ mol/L in all mice. We performed intracellular cytokine staining on CD4 T cells from spleens and kidneys at day 21 and confirmed that transferred polarized T cells had retained their polarity *in vivo* (Figure 3, H–I).

We confirmed the increased pathogenicity of T_H17 cells in a second experiment in which mice were sacrificed at day 14 after injection of T_H1 or T_H17 cells. In this experiment OT2 rather than OT2 $\times$ $RAG1^{-/-}$ cells were used, and two cell lines of each type (T_H1 or T_H17) were generated, with each mouse receiving one of these cell lines followed by an injection of peptide-conjugated Fab_2 fragments or unconjugated Fab_2 fragments as a control. The polarity of the injected cells is shown in Figure 4A. Glomerular CD4 $^{+}$ T cells and CD68 $^{+}$ macrophages, crescent formation, and interstitial CD4 $^{+}$ T cells were again greater in mice given T_H17 cells and peptide-conjugated Fab_2 as shown in Figure 4, B–F. In addition, interstitial macrophage numbers were higher in this group (Figure 4E). We again assessed glomerular neutrophil numbers, and these numbers were small with no

differences between groups (mean, <0.1 cells per GCS for all groups). In this experiment, albuminuria was measured at day 14 but was less than 50 μ g/mL (the detection limit of the assay used) in all mice. In this second experiment, we assessed absolute CD4⁺ T-cell numbers in spleens at day 14 (which we had not done in the first experiment in shown in Figure 4) and found no significant differences among any of the four groups (Figure 4G). This finding meant that the increased pathogenicity of T_H17 cells was not due to increased survival or proliferation. We also performed intracellular cytokine staining on CD4⁺ T cells from spleens taken at the end of this experiment and again confirmed that transferred CD4⁺ T cells retained their polarity (Figure 4H).

Discussion

These data established that crescentic glomerulonephritis could be induced by T_H17 but not T_H1 CD4⁺ T cells. Significant albuminuria was induced by CD4⁺ T cells that were sensitized *in vivo* in the initial experiment shown in Figure 2, but albuminuria was not significantly different from controls when *in vitro* sensitized T_H1 or T_H17 cells were injected. There are a number of possible reasons for this finding. In the initial experiment, we used intact whole IgG to plant ovalbumin peptide in the glomerulus, whereas Fab₂ fragments were used with *in vitro* polarized T cells. It is therefore possible that the additive effect of the antibody, which was not pathogenic on its own, contributed to the proteinuria. Alternatively, the *in vitro* polarized T cells may have been of a lower pathogenicity than the *in vivo* sensitized cells. An additional possibility is that full expression of disease with proteinuria requires both T_H1 and T_H17 subsets. In keeping with our data, proteinuria may be mild in other models in which crescentic glomerulonephritis is not induced by immune complexes, at least in C57BL/6 mice. For example, significant crescent formation may be seen in antineutrophil cytoplasmic antibody vasculitis models in C57BL/6 mice in association with minimal proteinuria. Despite this limitation, histologic crescentic glomerulonephritis was clearly induced by T_H17 and not T_H1 cells. Recent data have shown that in certain circumstances CD4 T-cell subsets have plasticity and that T_H17 cells may convert to T_H1 cells.²⁷ Therefore, it was important to show that in this system transferred CD4 T cells had retained polarity in both the spleen (Figure 3, H-I, and Figure 4H) and kidney (Figure 3, H-I).

The precise mechanism by which T_H17 cells localize to the glomerulus to induce glomerulonephritis is not yet defined. Previous work has shown that major histocompatibility complex class II expression on both leukocytes and parenchymal renal cells is required for glomerulonephritis.²⁸ We could therefore speculate that our peptide-Fab₂ conjugate is taken up by both renal antigen-presenting cells and non-bone marrow-derived renal cells. This leads to localization of T_H17 cells in the kidney with the release of proinflammatory cytokines and the development of glomerulonephritis. A range of proinflammatory cytokines are produced by T_H17 cells and act on both immune and nonimmune cells. These cytokines include IL-21, IL-22, IL-17A, IL-F, tumor necrosis factor, IL-6, and granulocyte-macrophage colony-

stimulating factor.²⁹ The current study has not defined which of these cytokines lead to crescentic glomerulonephritis, although it is likely to be a combination of several. It does not appear to require a prominent neutrophil influx because this was not seen. It is not clear why T_H17 but not T_H1 cells localized to the glomerulus in our model. Because all of the transferred CD4⁺ cells expressed the OT2 T-cell receptor, they had the same antigen specificity and the numbers of T_H1 and T_H17 cells in the spleens of recipient mice were similar at day 14 (Figure 4G). In a recent study, IFN- γ -secreting CD4 T cells were isolated from renal tissue in the nephrotoxic nephritis model.³⁰ It is possible that inflammation (initiated by immune complexes in the nephrotoxic nephritis model) is necessary to provide the chemokine secretion required to retain T_H1 cells in the kidney. This requirement may not be present or as stringent for T_H17 cells.

Our results contrast with a recent report in which both T_H1 and T_H17 cells induced mild proteinuria and only T_H1 cells caused crescent formation.¹⁹ A similar experimental system was used, but there were some differences. A monoclonal antibody with specificity for the Goodpasture antigen was used to plant whole ovalbumin protein on the GBM. In this previous report, the effect of unconjugated antibody, together with T_H1 or T_H17 cells, on histologic changes or proteinuria was not assessed as a control. Activated OT2 T cells will proliferate when given to RAG1^{-/-} mice even in the absence of antigen. This effect could cause a systemic cytokine release that, combined with a nonpathogenic dose of unconjugated GBM-binding monoclonal antibody, could induce proteinuria, which may have been of a similar magnitude to the proteinuria seen in mice given ovalbumin-conjugated monoclonal antibody and polarized T cells. The differences in antigen preparation and targeting strategy in this previous report may also be relevant. The binding sites of the antibodies within the kidney would have been different, with a broader localization for our polyclonal antibody compared with the monoclonal antibody. In addition, the use of whole monoclonal antibody may have allowed uptake and presentation through Fc and complement receptor-mediated mechanisms, and this would not have been the case with our Fab₂ fragment. Therefore, the OT2 peptide may have been targeted to different cells. Although the IgG1 monoclonal used was not itself pathogenic, it may have had a synergistic effect with the CD4⁺ T cells because it would have bound to macrophage Fc γ RIII and hence could have contributed to disease.

Whatever the reasons for the differences among these studies, together they show that both T_H1 and T_H17 T cells are able to induce crescentic glomerulonephritis under appropriate experimental conditions. Further work will be required to explore the interactions of these CD4⁺ T-cell subsets and to precisely define the mechanisms by which they induce disease.

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