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Local macrophage proliferation in the pathogenesis of glomerular crescent formation in rat anti-glomerular basement membrane (GBM) glomerulonephritis

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SUMMARY

Glomerular crescent formation is a feature of aggressive forms of glomerulonephritis. The conventional view of crescent formation within Bowman's space involves proliferation of parietal epithelial cells and the recruitment of blood monocytes. However, the potential role of local macrophage proliferation in this process has not been investigated. The current study examines macrophage proliferation within Bowman's space on the basis of expression of the proliferating cell nuclear antigen (PCNA) in a rat model of crescentic glomerulonephritis (accelerated anti-GBM disease). $ED1^+$ macrophages accounted for 42% of cells within early cellular crescents, and 38% of these crescent macrophages were proliferating on the basis of PCNA expression. Macrophages became the dominant cell population in advanced cellular and fibrocellular crescents (64–71%), and there was a significant increase in the level of macrophage proliferation, with 62% and 67% of $ED1^+$ macrophages expressing the PCNA, respectively. This high level of macrophage proliferation was confirmed by incorporation of bromodeoxyuridine and the presence of mitotic figures within crescents. Indeed, macrophages accounted for 73% of all proliferating cells within advanced and fibrocellular crescents. Macrophage proliferation within Bowman's space was a local event, as shown by a lack of proliferating monocytes in the circulation, the presence of mitotic figures within crescents and a reciprocal relationship between the numbers of $ED1^+PCNA^+$ cells within Bowman's space compared with that in the capillary tuft during the progression from early to advanced and fibrocellular crescents. In conclusion, this study has changed the conventional view of the pathogenesis of crescent formation in glomerulonephritis with the demonstration of substantial local macrophage proliferation within Bowman's space. It is proposed that local proliferation is a major mechanism of macrophage accumulation within crescents and plays an important role in the progression of epithelial-dominated early cellular crescents to macrophage-dominated advanced and fibrocellular cellular crescents.

Keywords macrophage proliferation crescent fibrosis glomerulonephritis

INTRODUCTION

Glomerular crescent formation is a prominent feature of rapidly progressive glomerulonephritis, indicating severe glomerular damage and being a diagnostic and prognostic marker in this severe form of the disease. It is now widely recognized that cellular crescents are formed by proliferation of parietal epithelial cells and the recruitment of blood monocytes [1–4]. Macrophage accumulation at sites of inflammation has been conventionally viewed as essentially a process of blood monocyte recruitment, with little or no contribution from local monocyte/macrophage proliferation [5]. However, this conventional view of macrophage accumulation has been changed by recent studies in which significant numbers of proliferating macrophages have been demonstrated within a variety of inflammatory lesions [6–10]. Indeed, the high numbers of dividing macrophages seen in experimental models of acute renal allograft rejection and Goodpasture's syndrome have shown that local proliferation is a major mechanism of macrophage accumulation in severe inflammatory reactions [6,7]. Furthermore, the number of proliferating macrophages was found to give an excellent correlation with the development of renal injury in accelerated anti-GBM glomerulonephritis in the rat [11]. This led to the hypothesis that local macrophage proliferation is a mechanism of amplifying local cellular immune responses, resulting in severe tissue damage. However, the contribution of local macrophage proliferation to the pathogenesis of glomerular crescent formation, a feature of severe glomerular damage, is unknown. This issue has been addressed by

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examining macrophage proliferation within Bowman's space during the development of crescentic glomerulonephritis in the rat using expression of the proliferating cell nuclear antigen (PCNA) [12,13], and incorporation of the thymidine analogue 5-bromo-2-deoxyuridine (BrdU) as markers of cell proliferation.

MATERIALS AND METHODS

Experimental crescentic glomerulonephritis

Inbred male Sprague-Dawley rats (150–200 g) were obtained from Monash Animal Services (Melbourne, Australia). Anti-GBM glomerulonephritis was induced as previously described [11,14]. Briefly, animals were immunized subcutaneously with 5 mg normal rabbit IgG in Freund's complete adjuvant (FCA) and injected intravenously with 10 ml/kg body weight rabbit anti-rat GBM serum (12·5 mg/ml IgG) 5 days later (termed day 0). Groups of six animals were killed on days 1, 7, 14, 21 and 28. In addition, two animals in each group were injected intraperitoneally with 50 mg/kg BrdU 3 h before being killed. A group of six normal animals was used as a control.

Histopathology

Tissues were fixed in formalin and 4-μm paraffin sections were stained with haematoxylin and eosin (H–E) or periodic acid-Schiff (PAS). Glomerular crescent formation was scored as previously described [4]. Briefly, the percentage of glomeruli with crescent formation was assessed by examining 50–100 glomeruli per animal on PAS-stained paraffin tissue sections. Glomerular crescents were classified into four categories as follows: (i) early cellular crescents were defined as cellular crescents with less than three layers of cells or filling less than half of the cross-section of Bowman's space; (ii) advanced cellular crescents contained three or more layers of cells or filled more than half of Bowman's space; (iii) fibrocellular crescents were defined as cellular crescents containing fibroblasts and some collagen; and (iv) fibrous crescents had a fibrous appearance and relatively few cells. Fibrous crescents were excluded from this study. In addition, Bowman's capsule disruption was determined in PAS-stained paraffin tissue sections.

Antibodies

The following MoAbs were used in this study: ED1, monocytes and tissue macrophages, anti-lysosomal CD68-like antigen [15,16]; ED2, subset of mature tissue macrophages [15]; ED3, subset of mature tissue macrophages [15]; PC-10, anti-proliferating cell nuclear antigen [17]; and M744, anti-bromodeoxyuridine, obtained from Dako Ltd (Glostrup, Denmark). Goat anti-mouse IgG, peroxidase-conjugated goat anti-mouse IgG, mouse peroxidase anti-peroxidase (PAP) complexes and alkaline phosphatase anti-alkaline phosphatase (APAAP) complexes were obtained from Dako.

Immunohistochemistry

Two- and three-colour immunohistochemistry staining was performed using a newly described microwave-based technique [18]. Briefly, paraffin sections were dewaxed in Bio-Clear (Midway Scientific, Melbourne, Australia), rehydrated through graded alcohols and treated with microwave oven heating for 2 × 5 min in 0·01 M sodium citrate pH 6·0 at 2450 MHz and 800 W power output. This step enhanced antibody detection of the PCNA and the cytoplasmic ED1 (CD68) antigen. In addition, microwave oven heating denatured DNA to facilitate antibody access to incorporated BrdU. Next, sections were preincubated with 10% fetal calf serum (FCS) and 10% goat serum in PBS for 20 min, drained and labelled with the ED1 MoAb at a 1:300 dilution for 60 min. Endogenous peroxidase was inactivated by incubation in 0·3% H_2O_2 in methanol, and then sections were labelled with peroxidase-conjugated goat anti-mouse IgG followed by mouse PAP and developed with diaminobenzidine to produce a brown colour. Sections were microwave-heated a second time to denature bound immunoglobulin molecules which prevents antibody cross-reaction. This was followed by a second preincubation step as above and incubation with either PC-10 MoAb (1:1000 dilution), or M744 MoAb (1:100 dilution). After washing in PBS, sections were labelled sequentially with goat anti-mouse IgG and mouse APAAP and developed with Fast Blue BB Base (Sigma Chemical Co., St Louis, MO). Sections were counterstained with PAS reagent without haematoxylin and coverslipped in an aqueous mounting medium. Some sections labelled with ED1 and PCNA MoAbs were microwaved a third time and then labelled with the M744 MoAb followed by goat anti-mouse IgG, then mouse APAAP and developed with Fast Red. In addition, cell spots were prepared from peripheral blood mononuclear cells (PBMC) by Ficoll-gradient centrifugation at the time of sacrifice. These cell spots were double-stained with ED1 and PC10 or ED1 and BrdU MoAbs as described above. As a negative control, an isotype-matched irrelevant MoAb (73.5; mouse anti-human CD45R) was used in place of one or both primary antibodies which produced no signal.

Quantification of proliferating cells

Cells within early cellular, advanced cellular and fibrocellular crescents were scored in paraffin sections labelled with either ED1 and PC-10 or ED1 and M744 MoAbs counterstained with PAS (minus haematoxylin) as previously described [4]. At least 20 glomerular cross-sections containing crescents were scored per animal. This enabled over 100 of each crescent type to be scored in total. The total number of cells within each crescent cross-section was counted and divided into $ED1^+$ and $ED1^-$ cells. At the same time, the number of nucleated $ED1^+$ macrophages was counted and each cell was assessed for nuclear anti-PCNA ($ED1^+PCNA^+$) or anti-BrdU ($ED1^+BrdU^+$) staining. In addition, the total number of $PCNA^+$ and $BrdU^+$ cells and the number of $ED1^-PCNA^+$ and $ED1^-BrdU^+$ cells within cross-sections of glomerular crescents was assessed. Data are presented as the mean ± s.e.m. per glomerular crescent cross-section (gcs).

In glomeruli where cells were counted within cellular crescents, the number of $ED1^+$ and $ED1^+PCNA^+$ cells within the glomerular tuft was also scored in order to compare macrophage proliferation in the two areas. Data are presented as mean ± s.e.m. per gcs.

Statistical analysis

Differences in the number of $ED1^+$ or $ED1^+PCNA^+$ cells in glomerular capillary tufts and crescents were analysed by an unpaired two-sided Student's *t*-test. In addition, differences in the number of $ED1^+PCNA^+$ and $ED1^-PCNA^+$ cells in different categories of glomerular crescents was compared by an unpaired two-sided Student's *t*-test.

RESULTS

Development of glomerular crescents

Early cellular crescents were first detected on day 7 of anti-GBM glomerulonephritis, being present in 14 ± 3·2% (range 5–29%) of

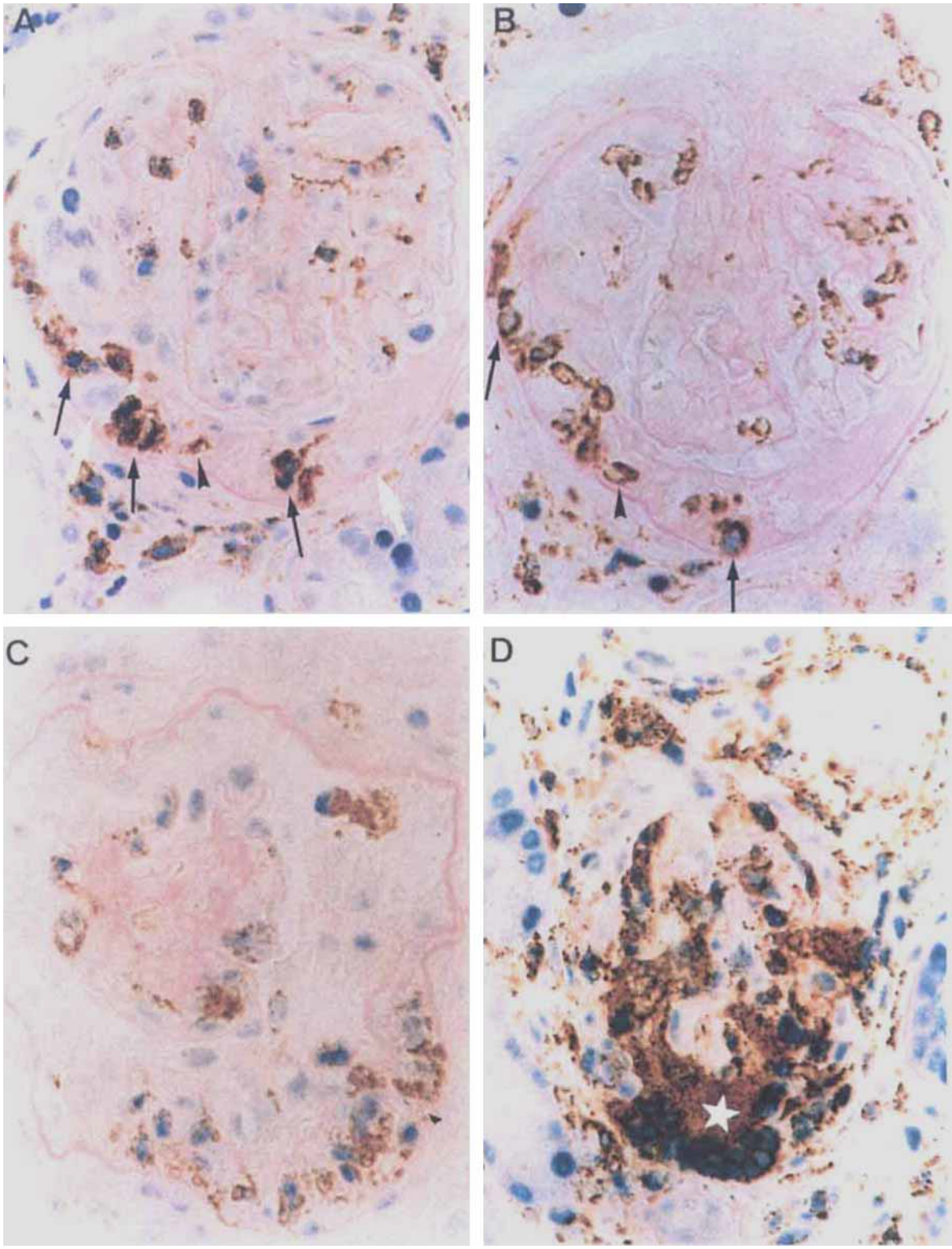

Fig. 1. Double immunohistochemistry showing macrophage proliferating cell nuclear antigen (PCNA) expression and 5-bromo-2-deoxyuridine (BrdU) incorporation within early and advanced cellular crescents in rat anti-GBM glomerulonephritis. Brown cytoplasmic staining indicates $ED1^+$ macrophages, while blue nuclear staining indicates $PCNA^+$ or $BrdU^+$ cells. Examples of $ED1^+PCNA^+$ or $ED1^+BrdU^+$ (arrows) and $ED1^+PCNA^-$ (arrowheads) macrophages are indicated. (A) An early cellular crescent from day 7 of disease, showing similar numbers of proliferating macrophages ($ED1^+PCNA^+$, arrows) and proliferating glomerular visceral and parietal epithelial cells ($ED1^-PCNA^+$, white arrows) within the crescent. (B) Macrophage proliferation was confirmed by BrdU labelling (arrows) in a serial section from the same early cellular crescent as seen in (A). (C) An advanced cellular crescent from day 14 of disease. $ED1^+$ macrophages are the major cell type within the crescent and many of the macrophages are proliferating ($ED1^+PCNA^+$ cells). (D) An advanced cellular crescent from day 21 of disease, showing severe glomerular and Bowman's capsular destruction and large numbers of proliferating macrophages, including multinucleated giant cells (*), within the crescent. Sections were counterstained with periodic acid-Schiff (PAS) without haematoxylin (×400).

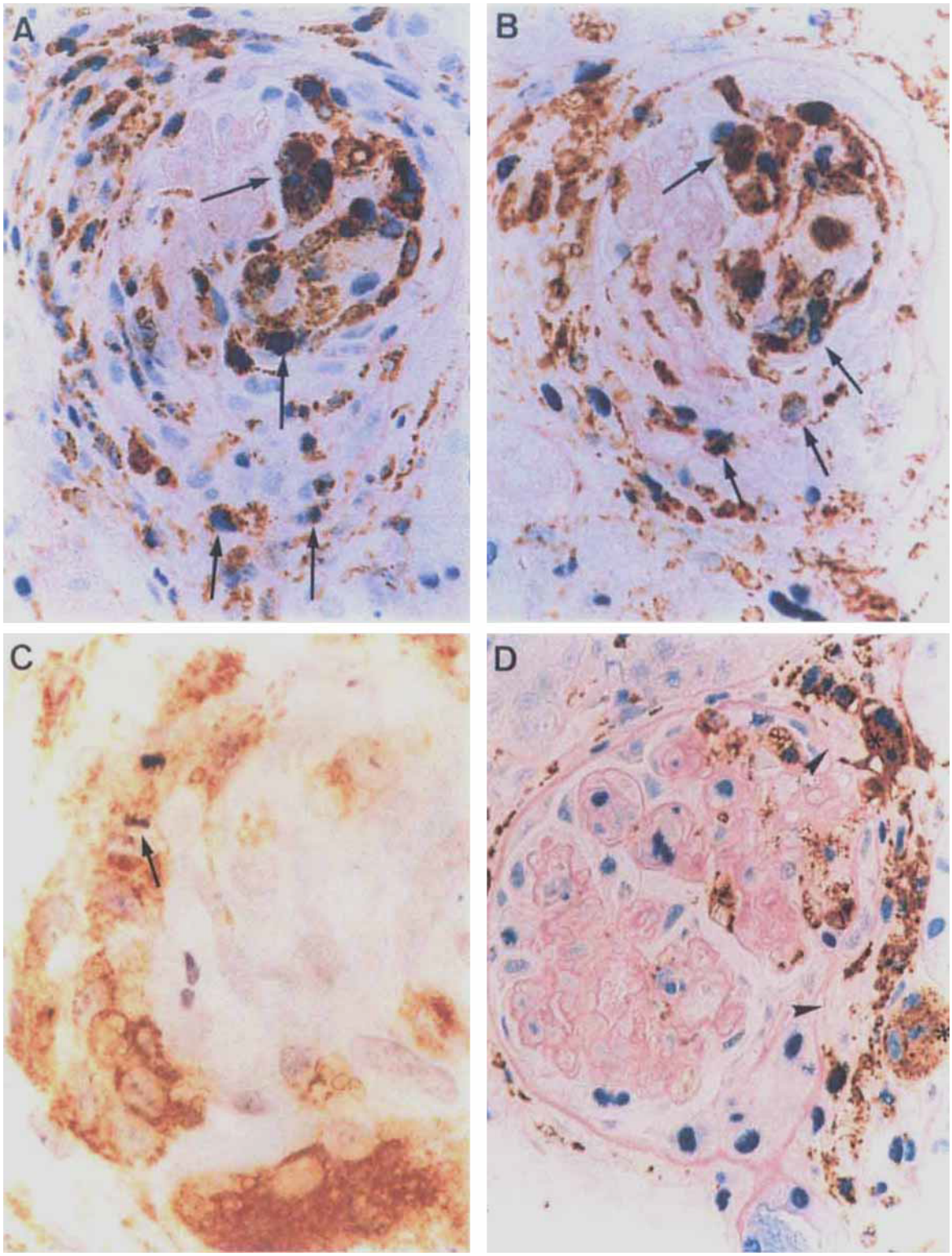

Fig. 2. Double immunohistochemistry staining showing local macrophage proliferation and cell division within advanced and fibrocellular crescents and in the periglomerular area in rat anti-GBM glomerulonephritis. Brown cytoplasmic staining indicates $ED1^+$ macrophages, while blue nuclear staining indicates proliferating cell nuclear antigen $(PCNA)^+$ or 5-bromo-2-deoxyuridine $(BrdU)^+$ cells. (A) A fibrocellular crescent from day 28 of disease, showing numerous $ED1^+ PCNA^+$ macrophages (arrows) within an area of glomerular segmental proliferation and within a fibrocellular crescent featuring extensive Bowman's capsular disruption. (B) Many $ED1^+ BrdU^+$ S-phase proliferating macrophages (arrows) are found within the glomerular segmental proliferative lesion, but only a few $ED1^+ BrdU^+$ macrophages seen within fibrocellular crescent (arrows) compared with PCNA staining in a serial section to that seen in (A). (C) An advanced cellular crescent containing numerous $ED1^+$ macrophages, including one $ED1^+$ crescent cell exhibiting a telophase mitotic figure (arrow). (D) Day 21 of disease, showing the presence of periglomerular macrophage proliferation, including multinucleated giant cell formation (*), adjacent to an area of Bowman's capsular disruption (arrowheads). Sections were counterstained with periodic acid-Schiff (PAS) without haematoxylin (×400).

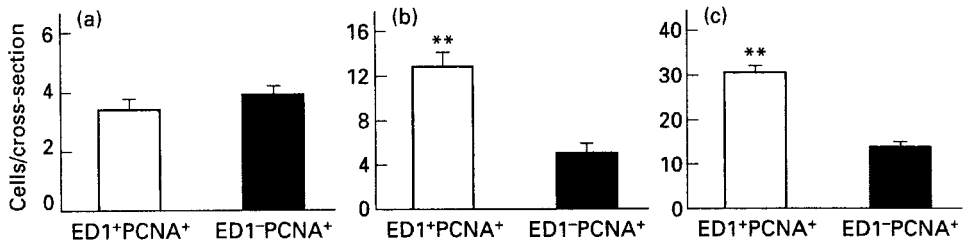

Fig. 3. Quantitative analysis of proliferating cells within early (a), advanced (b) and fibrocellular crescents (c) in rat anti-GBM glomerulonephritis. The number of proliferating macrophages ($ED1^+PCNA^+$ cells, □) and non-macrophage proliferating cells ($ED1^-PCNA^+$ cells, ■) in more than 100 of each type of crescent scored from animals over the entire disease course (days 7, 14, 21 and 28). Data are expressed as mean ± s.e.m. $^{**}P<0{\cdot}001$.

glomeruli examined. There was a significant increase in the percentage of glomeruli exhibiting crescent formation as the disease progressed, with 30 ± 2·5%, 58·5 ± 4·9% and 63 ± 3·5% of glomeruli with crescent formation on days 14, 21 and 28, respectively. During this time course there was a change in the nature of crescents observed. While all crescents seen on day 7 were of the early cellular type, by day 14, 40–60% of crescents were of the advanced cellular type. Most crescents seen on days 21 and 28 (60–80%) were of the advanced cellular or fibrocellular type. Histologically, early cellular crescents exhibited a large amount of H–E- and PAS-stained plasmic fluid within Bowman's space, there was minor damage within the glomerular capillary tuft, and Bowman's capsule remained intact. The development of large advanced cellular and fibrocellular crescents was associated with severe glomerular damage, including focal segmental necrosis and sclerosis and extensive disruption of Bowman's capsule.

Macrophage accumulation and proliferation during glomerular crescent formation

Macrophages ($ED1^+$ cells) accounted for 42% of total cells within early cellular crescents. With the development of advanced and fibrocellular crescents, $ED1^+$ macrophage became the major cell type within crescents and made up 71% and 64% of total crescent cells, respectively. Examples of $ED1^+$ macrophages within crescents are shown in Figs 1 and 2.

Using PCNA expression as a marker of cell proliferation, significant numbers of $ED1^+PCNA^+$ proliferating macrophages were seen in early cellular crescents (Figs 1A and 3a). Indeed, proliferating cells accounted for 38·5 ± 2·1% of the total $ED1^+$ macrophage population in early cellular crescents (Fig. 4a). However, most proliferating cells within early cellular crescents had an $ED1^-$ phenotype and appeared to be predominantly glomerular parietal and visceral epithelial cells (Figs 1A and 3a). There was a significant increase in the absolute numbers of $ED1^+$ macrophages as crescents developed from an early to an advanced phenotype (Figs 1C and 4b). There was also a marked increase in the percentage of macrophages which were proliferating ($ED1^+PCNA^+$ cells) in advanced cellular crescents. Indeed, at this stage $ED1^+PCNA^+$ macrophages accounted for the majority of $PCNA^+$ cells within advanced cellular crescents (Fig. 3b). Fibrocellular crescents contained a greater absolute number of $ED1^+$ macrophages compared with advanced cellular crescents, and the percentage of proliferating ($ED1^+PCNA^+$) cells within this population remained very high at 66·6 ± 2·2% (Figs 2A and 3c). Of interest was the observation of strong PCNA and CD68 expression in multinucleated giant cells present within some advanced cellular crescents, resulting in granulomatous crescent formation and Bowman's capsular destruction (Fig. 1D). However, it should be pointed out that these high levels of macrophage proliferation were restricted to areas of focal tissue damage, with relatively little macrophage proliferation seen in areas of intact tissue.

DNA incorporation of the thymidine analogue BrdU, injected 3 h before sacrifice, was used as an alternative method of assessing cellular proliferation. Double immunohistochemistry showed that all $BrdU^+$ cells were also $PCNA^+$. The number of $ED1^+BrdU^+$ macrophages accounted for ≈30–40% of the $ED1^+PCNA^+$ cells within early and advanced cellular crescents, consistent with BrdU incorporation marking cells during S-phase while PCNA is expressed during G_1, S, G_2 and some parts of M phases of the cell cycle [12,13]. An example comparing PCNA and BrdU labelling is shown in serial sections of an early cellular crescent in Fig. 1A,B. However, there was a relative reduction in BrdU staining of macrophages and non-macrophage ($ED1^-$) cells in fibrocellular crescents compared with the number of $PCNA^+$ cells (Fig. 2A,B). Indeed, this relative reduction in the number of

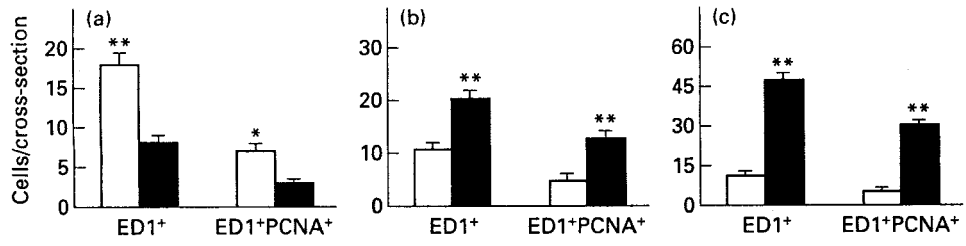

Fig. 4. Quantitative analysis of the total number of macrophages ($ED1^+$ cells) and proliferating macrophages ($ED1^+PCNA^+$ cells) within early (a), advanced (b) and fibrocellular crescents (c) in rat anti-GBM glomerulonephritis. Both the glomerular tuft (□) and crescent (■) were scored in each case. Data are pooled from the entire disease course (days 7, 14, 21 and 28) and expressed as the mean ± s.e.m. $^*P<0{\cdot}05$; $^{**}P<0{\cdot}001$.

$BrdU^+$ cells was also seen in fibrotic areas within the tubulointerstitium, while other non-fibrotic areas in glomeruli and the tubulointerstitium demonstrated the usual 30–40% ratio of $BrdU^+$ to $PCNA^+$ cells. This finding could relate to the lack of blood supply to fibrotic areas and thus reduced access of BrdU to proliferating cells, or it may reflect some fundamental change in cell biology in fibrotic areas, with cells undergoing either a process of differentiation or apoptosis while in the G_1 phase ($PCNA^+ BrdU^-$) of the cell cycle.

As further evidence that macrophages were completing cell division within crescents, the presence of mitotic figures in $ED1^+$ cells was observed. An example of an $ED1^+$ macrophage in telophase in an advanced cellular crescent is shown in Fig. 2C, and this formally demonstrates macrophage division within cellular crescents in this disease model.

Proliferating macrophages in crescents and in the capillary tuft had an $ED1^+ ED2^- ED3^-$ phenotype, demonstrating that they are recently recruited blood monocytes rather than resident tissue macrophages which express the ED2 and/or ED3 antigens [17]. No PCNA expression or BrdU incorporation was detected in $ED1^+$ blood mononuclear cells obtained at the time of sacrifice in either normal or diseased animals. Therefore, proliferation of $ED1^+ ED2^- ED3^-$ cells was a local event within the kidney.

Comparison of macrophage proliferation within the glomerular tuft with that in crescents

The relationship between macrophage proliferation in the glomerular tuft and in Bowman's space was examined. The number of $ED1^+ PCNA^+$ cells within early cellular crescents was much less than that present in the capillary tuft, although the percentage of $ED1^+$ macrophages exhibiting PCNA expression was very similar in the two populations (Fig. 4a). This pattern changed dramatically in glomeruli featuring advanced and fibrocellular crescents. There was a reduction in the number of total macrophages and proliferating macrophages within the tuft, whereas there was a significant increase in the number of total macrophages and proliferating macrophages within crescents (Fig. 4b,c). This reciprocal relationship suggests that proliferating macrophages within advanced and fibrocellular crescents are not simply derived by passive migration of cells from the glomerulus.

Macrophage proliferation and Bowman's capsule rupture

Disruption of Bowman's capsule integrity is a feature of this model of crescentic glomerulonephritis which is associated with activated periglomerular immune cells in what is most probably a DTH reaction [4]. Using PAS to stain Bowman's capsule basement membrane, early cellular crescents were shown to have an intact capsule. In contrast, disruption of Bowman's capsule was evident in > 50% of glomeruli with large advanced cellular crescents and in all glomeruli exhibiting fibrocellular crescents. A striking feature was that sites of rupture of Bowman's capsule were immediately adjacent to areas of focal periglomerular macrophage accumulation and proliferation, regardless of the presence or absence of glomerular crescent formation (Fig. 2D). Indeed, > 70% of $ED1^+$ macrophages within these periglomerular infiltrates adjacent to sites of rupture exhibited PCNA expression. In contrast, there was only minor macrophage accumulation or proliferation in periglomerular areas where Bowman's capsule remained intact (Figs 1 and 2). Interestingly, $ED1^+ PCNA^+$ multinucleated giant cells were present in periglomerular areas featuring a granulomatous response and extensive disruption of Bowman's capsule (Fig. 2D).

One feature associated with disruption of Bowman's capsule was a significant increase in the number of proliferating $ED1^-$ cells within advanced and especially fibrocellular crescents compared with early cellular crescents (Fig. 4). This presumably reflects the presence of proliferating fibroblasts and T cells which have entered Bowman's space through the ruptured basement membrane from the periglomerular area during the development of fibrocellular crescents (Fig. 2A,B).

DISCUSSION

Recent studies demonstrating high levels of macrophage proliferation within severe inflammatory lesions have changed the conventional view that macrophage accumulation at sites of inflammation operates through monocyte recruitment, with little or no contribution by local proliferation [5–10]. The current study has extended these findings to gain a new understanding of the pathogenesis of glomerular crescent formation in experimental glomerulonephritis. On the basis of these findings, it is postulated that macrophage proliferation occurs locally within Bowman's space and is a major mechanism of macrophage accumulation in the formation of cellular crescents and plays a key role in the progression of early cellular crescents to advanced and fibrocellular crescents. Evidence to support these conclusions is discussed below.

In this and previous studies of experimental anti-GBM glomerulonephritis no evidence has been found of monocyte proliferation within the circulation, demonstrating that the large numbers of proliferating macrophages seen within the kidney represent a local component of the aggressive inflammatory response in this disease [7,11]. The current study has, for the first time, shown that high levels of macrophage proliferation are present in Bowman's space during the process of early crescent formation and the subsequent development into advanced and fibrocellular crescents. The presence of proliferating macrophages within Bowman's space could arise from local proliferation in Bowman's space itself or it could result from migration of $ED1^+ PCNA^+$ cells from the glomerular tuft into Bowman's space. Two observations support the view that macrophage proliferation is a local event within Bowman's space itself. First, the number of $ED1^+ PCNA^+$ cells within Bowman's space increased markedly in the progression from early to advanced and fibrocellular crescents, while there was a reciprocal decrease in the number of $ED1^+ PCNA^+$ cells within the capillary tuft in these glomeruli, arguing against migration of $ED1^+ PCNA^+$ cells from the glomerular tuft into Bowman's space. Second, macrophages were shown to undergo cell division within cellular crescents by virtue of the presence of macrophages exhibiting mitotic figures. Indeed, mitotic figures in $ED1^+$ macrophages were easier to locate within crescents than within the glomerular tuft.

The large number of proliferating macrophages seen within glomerular crescents, whether assessed by PCNA expression or BrdU incorporation, would have been expected to give rise to a far larger number of total macrophages than that actually observed. This has two important implications. First, local proliferation is clearly a major mechanism of macrophage accumulation within Bowman's space in the formation and development of crescents. Second, there must be a very high level of macrophage turnover within Bowman's space. This is easily envisaged if many of the progeny of dividing cells become detached and released into the urine. Indeed, this is supported by the presence of many $ED1^+ PCNA^+$ macrophages within the tubular luminal space in cellular casts (data not shown).

Cell proliferation clearly plays a major role in the pathogenesis of crescent formation in this model of glomerulonephritis. Most proliferating cells within early cellular crescents were parietal epithelial cells, suggesting that one of the earliest events in the process of crescent formation is the stimulation of epithelial cell proliferation. In contrast, the development from early to advanced and fibrocellular crescents involves a substantial increase in the number of proliferating macrophages, and macrophages become the dominant cell type within these crescents. This development from a stage of predominantly epithelial cell proliferation to one of predominantly macrophage proliferation probably accounts for some of the controversy in the literature regarding whether parietal epithelial cells or macrophages are the main component of cellular crescents [19,20]. In addition to the change of cellular composition as crescents develop from an early to an advanced or fibrocellular type, there is also a loss of Bowman's capsule integrity in this process. This is a crucial step in crescent pathogenesis, as it facilitates the entry of periglomerular fibroblasts and T cells into Bowman's space, which results in the development of fibrosis [4,21,22].

In previous studies of this disease model we have shown that Bowman's capsule rupture is invariably associated with focal periglomerular infiltrates of activated T cells and macrophages, irrespective of the presence of crescents within Bowman's space. Further evidence to demonstrate that periglomerular leucocytes can mediate Bowman's capsule rupture through a DTH mechanism comes from a recent study of interstitial nephritis induced in rats by active immunization with autologous Tamm–Horsfall protein. In this model, rupture of Bowman's capsule occurred in the absence of glomerular inflammation, but in association with periglomerular accumulation of T cells and macrophages together with antigen and expression of macrophage migration inhibitory factor [23]. These finding have been extended in the current study with the observation that proliferating macrophages within the periglomerular area were almost exclusively restricted to sites of Bowman's capsule rupture and that proliferating macrophages were intimately associated with multinucleated giant cells which were observed at some sites of rupture. As yet the function of such marked macrophage proliferation at these sites of damage is unclear. One possibility is that it is simply a mechanism of local amplification of the cellular immune response which results in severe tissue damage. A second possibility is that the proliferating macrophages may have a significant, or even enhanced, ability to directly mediate tissue damage. Indeed, macrophage proliferation appears to play an important role in multinucleated giant cell formation within granulomatous lesions in this model [24].

One issue arising from this study is what drives local macrophage proliferation within Bowman's space. As yet there is no clear answer, although one obvious candidate is macrophage colony-stimulating factor (M-CSF), a molecule which is both a chemoattractant and a growth factor for monocyte/macrophages [25]. Indeed, increased glomerular M-CSF expression has been implicated in glomerular macrophage accumulation and renal injury in murine lupus nephritis, and the introduction of transfected tubular epithelial cells secreting M-CSF under the kidney capsule results in local macrophage accumulation and renal injury in autoimmune mice [26–28]. Another molecule which promotes macrophage accumulation at sites of inflammation and is a potent inducer of macrophage activation is macrophage migration inhibitory factor (MIF) [29–31]. We have observed *de novo* MIF protein expression by parietal and visceral epithelial cells prior to the formation of early cellular crescents on days 1 and 7 in rat anti-GBM glomerulonephritis, suggesting that this molecule could be important in the induction of macrophage accumulation within Bowman's space [32].

In conclusion, the findings of the current study have changed the conventional view of the pathogenesis of crescent formation. Using an experimental model of crescent glomerulonephritis, we have demonstrated the presence of substantial local macrophage proliferation in Bowman's space during crescent formation. The results suggest that local proliferation is a major mechanism of macrophage accumulation within crescents and plays an important role in the progression of early epithelial-dominated cellular crescents to macrophage-dominated advanced and fibrocellular cellular crescents. Thus, macrophage proliferation may be a suitable target for therapeutic intervention in crescentic glomerulonephritis.

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