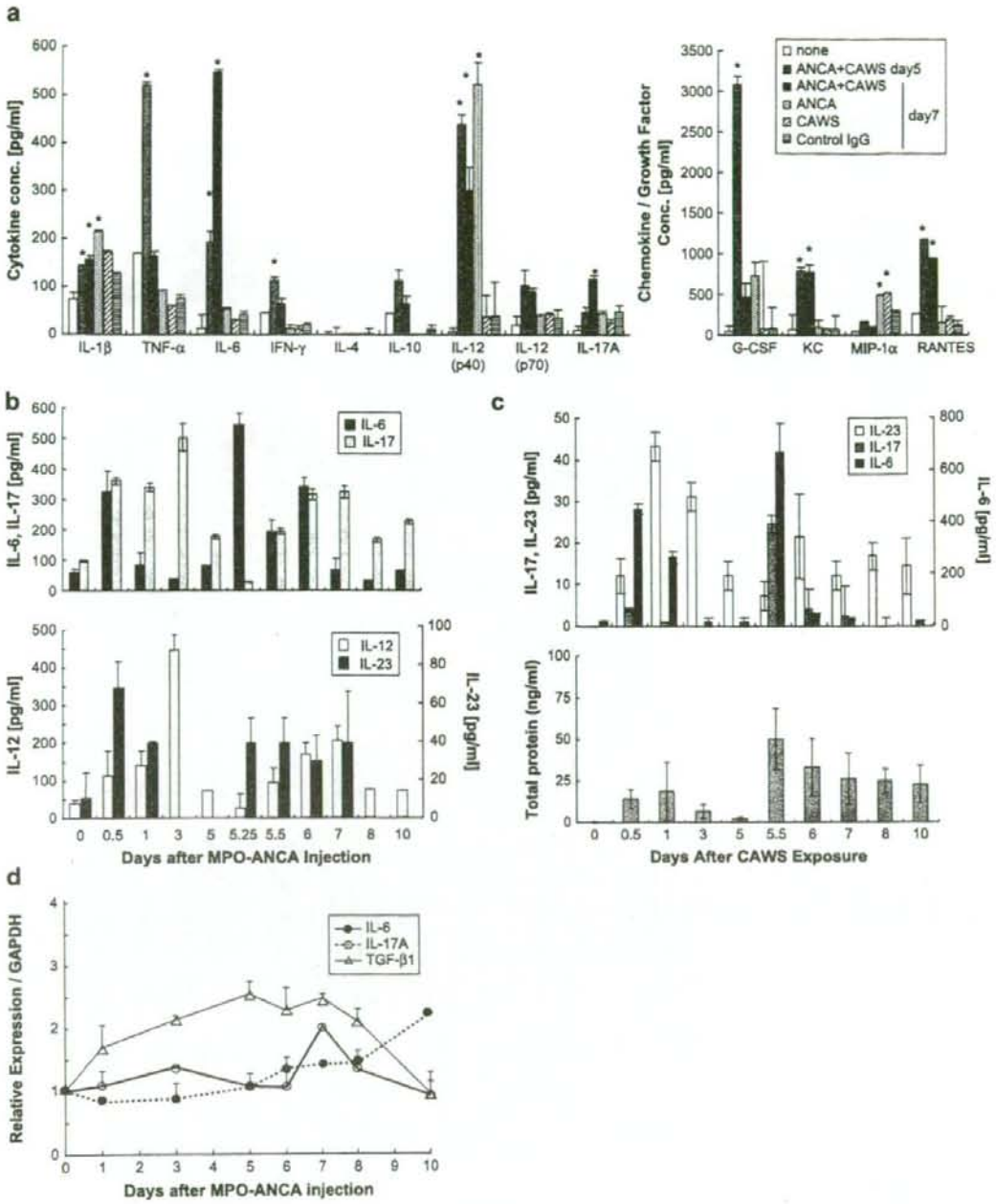

Fig. 2. Dramatically altered serum cytokine production by ANCA-induced systemic vasculitis mice. (a) The serum cytokine level of the mice treated with MPO-ANCA plus CAWS (on days 0, 5, and 7), with MPO-ANCA, with IgG, and with CAWS (on day 7) was measured by the Bio-Plex® multiple cytokine detection kit. The data are presented as the means ± SD of two independent serum samples from two separate experiments on a total of four mice. (b) Serum cytokine levels in ANCA-mediated systemic vasculitis. IL-6, IL-12p70(p35/p40), IL-17A, and IL-23(p19/p40) production in sera of the indicated time courses were measured as the means (pg/ml) ± SD of pooled data ($n = 8$–20 sera per each day) from four separate experiments. IL-6, IL-12p70, and IL-17A were detected with the Bio-Plex® multiple cytokine detection kit and IL-23 with IL-23p19-specific ELISA, respectively. (c) Top: IL-6, IL-17, and IL-23 levels in peritoneal lavage fluids. IL-17A (blue), IL-23(p19/p40; red, indicated on left scale) and IL-6 (green, indicated on right scale) production on the indicated days were measured as the means ± SD (ng/ml) of pooled data ($n = 4$–10 samples) from two separate experiments. Bottom: the total protein concentration in peritoneal lavage fluid was measured as the mean ± SD (ng/ml) of samples described above. (d) The transcription of *IL-6*, *IL-17A* and *TGF-β1* mRNA by CD4$^+$ T lymphocytes. *IL-6* (green), *IL-17* (blue), and *TGF-β1* (orange) mRNA production by splenic CD4$^+$ T cells (1×10^6) of mice treated with MPO-ANCA quantified by real-time PCR were shown as the mean ± SD of samples described above. Asterisk (*), the significance between the two indicated columns ($p < 0.05$).

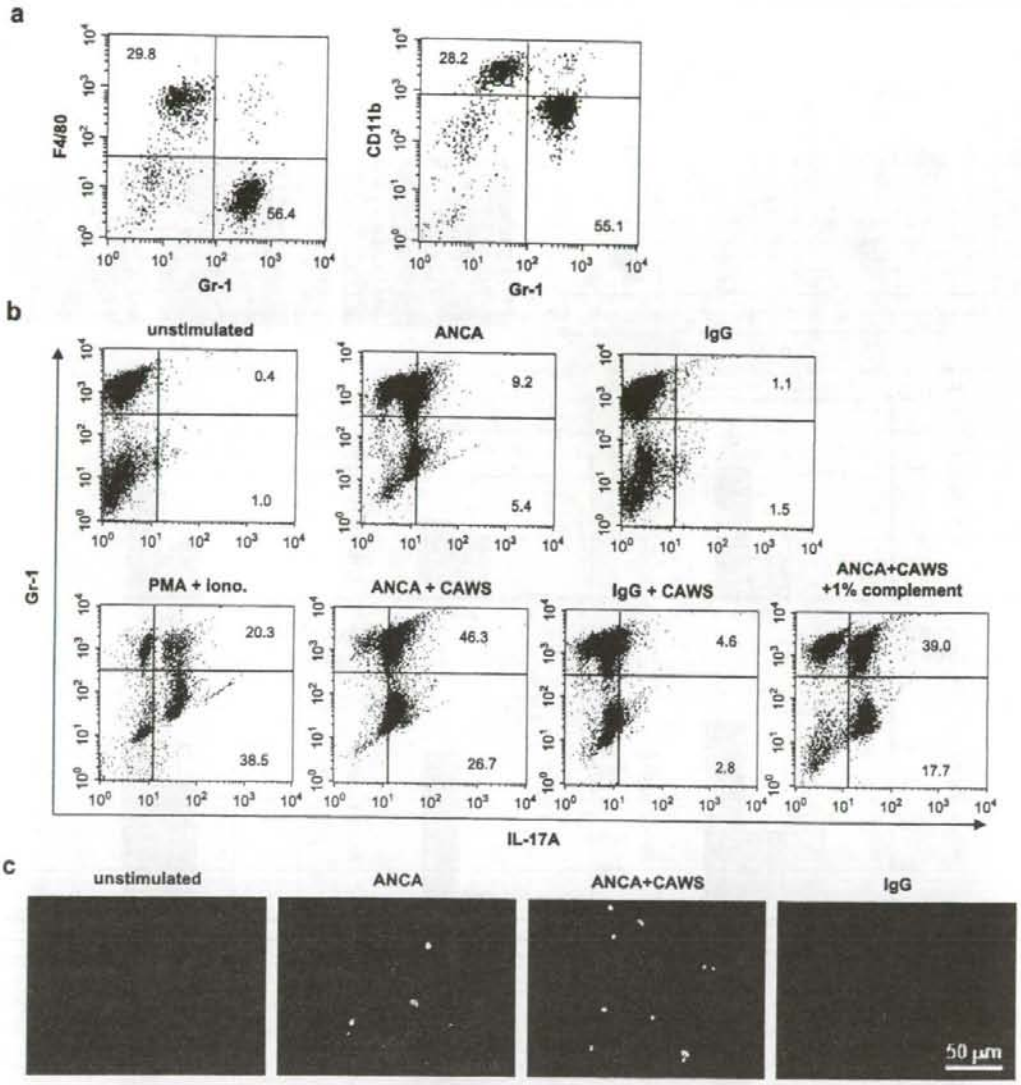

Fig. 3. ANCA induces IL-17A production in concert with CAWS mannoprotein by neutrophils *in vitro*. (a) A representative flow cytometric analysis showing the gating of acute inflammatory macrophages ($Gr\text{-}1^{int}$,$CD11b^{hi}$, $F4/80^{+}$) and acute inflammatory neutrophils ($Gr\text{-}1^{hi}$,$CD11b^{int}$, $F4/80^{-}$) in peritoneal exudate cells isolated from 8% casein activated peritoneum. (b) Intracellular cytokine staining of peritoneal casein-induced neutrophils. Peritoneal exudate cells stimulated with MPO-ANCA or control IgG (1 mg/ml), and CAWS (1 mg/ml), were stained with FITC anti-IL-17A, and PE anti-Gr-1, and the PerCP-Cy5.5 anti-F4/80 Abs. Acute inflammatory neutrophils were shown by gating out of F4/80-negative cells. (c) Immunohistochemical staining of neutrophils stimulated with ANCA. The neutrophils, stimulated with MPO-ANCA (1 mg/ml), in the presence of Brefeldin A and monensin for 6 h, were stained with FITC anti-IL-17A mAb. Original magnification: ×100, the bar indicates 100 μm.

chemokines and non-sensitive ones. The CAWS sensitive chemokines, MIP-1α, MIP-1β, RANTES, and KC have the ability to respond to CAWS, and MPO-ANCA enhanced their chemokine production. In contrast, CAWS has less effect on the expression of MCP-1 and eotaxin production by the peritoneal neutrophils, whereas the stimulation with only MPO-ANCA induced the chemokines (Fig. 4d). This could explain the result that stimulation with either CAWS or MPO-ANCA does not induce any systemic inflammation in mice [14], nevertheless they have the ability to allow neutrophils to infiltrate into the lung and kidney (see Fig. 1b). In addition, MPO translocation on the membrane surface was observed when neutrophils were activated, but only CAWS stimulation enhanced no MPO activation with MPO [14]. These results imply that stimulation with only CAWS is therefore insufficient to initiate neutrophil activation, but it does facilitate the neutrophil infiltration. Together, both MPO-ANCA and CAWS, which promotes IL-6 production by neutrophils, co-operatively induce the initial phase of neutrophil mediated systemic inflammation.

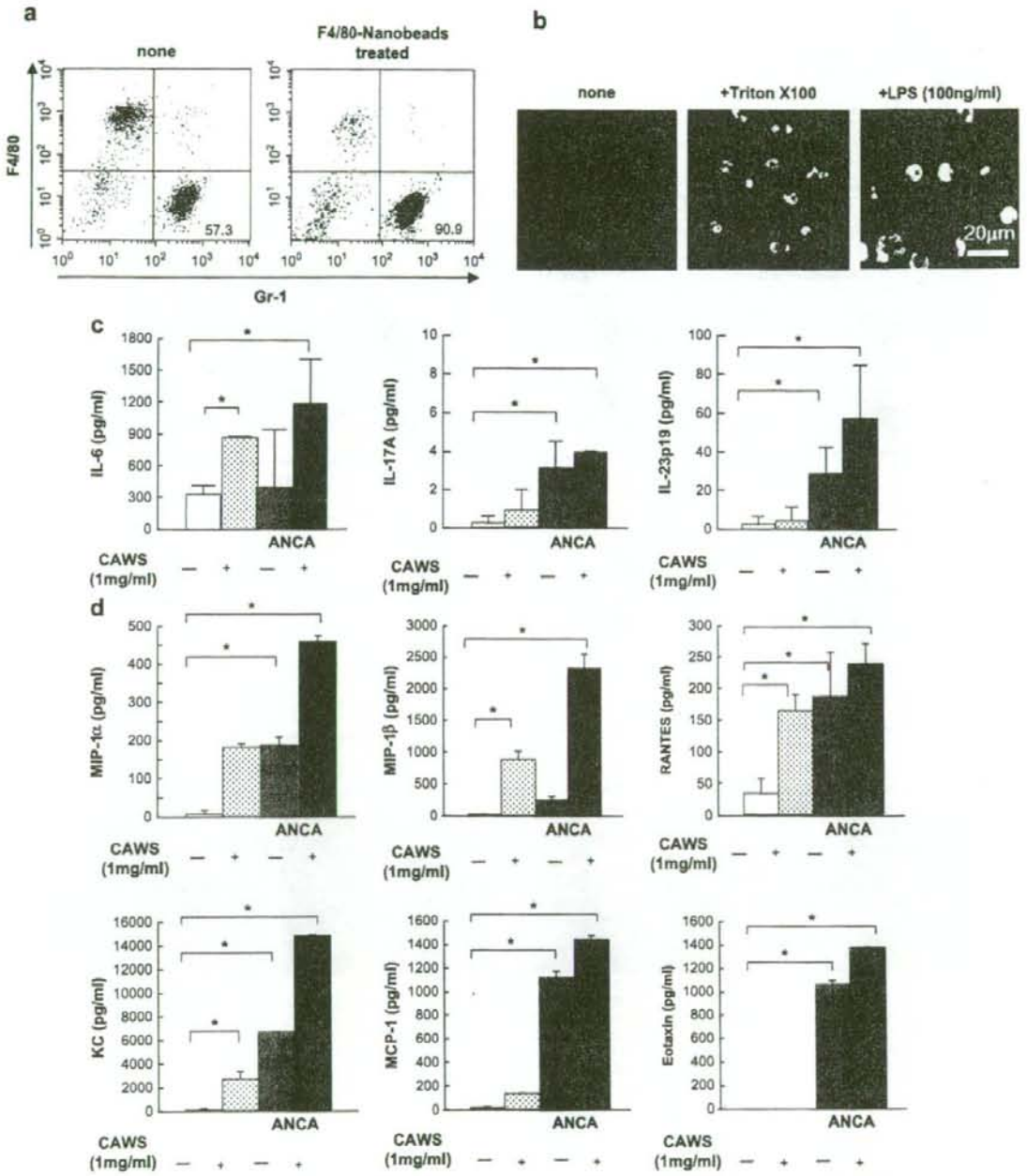

Fig. 4. Activated neutrophils produced multiple chemokines and chemokines by ANCA-dependent and independent mechanisms. (a) A representative flow cytometric analysis of freshly isolated peritoneal exudate cells treated with ThermaMax®-LA magnetic nanoparticles coated with streptavidin which pretreated with biotinylated anti-F4/80 Ab. After the depletion of F4/80-positive macrophages by magnetic nanoparticles, the neutrophils were stained with FITC anti-Gr-1, and PE anti-F4/80 Ab. (b) The activation of purified neutrophils was assessed by the surface expression of MPOs. The neutrophils were stained with QD640-conjugated anti-MPO mAb. Original magnification ×400; the bar indicates 20 μm. (c,d) The magnetic nanoparticles-purified neutrophils (1×10^6) were stimulated with MPO-ANCA(1 mg/ml) in the presence or absence of CAWS mannoprotein(1 mg/ml). Secreted cytokines (c) and chemokines (d) in culture sup after 24 h were measured as the means ± SD by the Bio-Plex® multiple cytokine detection system. Asterisk (*), a significant difference between the two indicated columns ($p < 0.01$).

Table 1
A comprehensive assay of cytokine production of activated neutrophils

	Unstimulated	CAWS	MPO-ANCA	MPO-ANCA + CAWS	IgG	IgG + CAWS
IL-1α	13.0 ± 1.4	35.6 ± 67.9	107.2 ± 44.6	154.2 ± 37.8	32.2 ± 5.3	111.7 ± 21.9
IL-1β	34.5 ± 1.0	44.3 ± 1.4	114.5 ± 3.5	142.0 ± 20.8	49.7 ± 3.9	98.8 ± 5.0
IL-2	7.8 ± 24.4	7.7 ± 27.2	6.6 ± 12.2	4.9 ± 9.6	4.7 ± 4.2	3.4 ± 3.5
IL-3	n.d.	n.d.	25.7 ± 8.1	23.0 ± 3.7	2.2 ± 4.2	8.0 ± 3.5
IL-4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IL-5	1.2.2 ± 3.5	0.6 ± 1.7	13.2 ± 27.5	17.8 ± 26.9	1.5 ± 1.1	6. 9 ± 6.3
IL-6	275.6 ± 44.36	1642.7 ± 302.3	580.5 ± 322.4	716.9 ± 73.2	86.1 ± 37.6	191.4 ± 17.2
IL-9	n.d.	27.4 ± 1.4	44.3 ± 2.8	61.3 ± 4.2	20.0 ± 1.7	49.6 ± 1.4
IL-10	n.d.	4.7 ± 2.8	49.1 ± 18.4	76.5 ± 2.8	13.1 ± 8.8	26.6 ± 1.8
IL-12p40	8.7 ± 19.1	13.1 ± 36.1	42.7 ± 37.1	55.5 ± 10.9	38.2 ± 26.5	35.1 ± 14.8
IL-12p70	n.d	2.6 ± 0.7	7.4 ± 0. 3	12.1 ± 9.9	3.0 ± 2.1	7.2 ± 0.7
IL-13	14.2 ± 0.7	29.2 ± 0.6	95.2 ± 0.0	124.1 ± 0.7	37.3 ± 1.1	101.7 ± 1.6
IL-17	0.28 ± 0..8	0.91 ± 0.0	3.17 ± 0.7	3.99 ± 0.5	1.44 ± 0.6	2.27 ± 1.4
Eotaxin	n.d.	n.d.	1066.1 ± 261.6	1380.3 ± 71.0	n.d.	n.d.
G-CSF	80.1 ± 10.5	378.0 ± 105.0	2007.9 ± 224.2	4356.6 ± 155.9	460.0 ± 24.4	2921.6 ± 116.0
GM-CSF	2.6 ± 1.0	6.4 ± 3.5	107.1 ± 119.4	179.3 ± 83.0	8.8 ± 1.4	22.6 ± 14.1
IFN-γ	n.d.	0.8 ± 0.7	2.6 ± 4.9	3.5 ± 1.7	n.d.	2.8 ± 0.0
KC	698.3 ± 43.4	2638.7 ± 680.9	6690.3 ± 148.5	14790.0 ± 115.6	3015.7 ± 41.2	6103.8 ± 48.4
MCP-1	83.8 ± 21.2	134.2 ± 38.9	1133.7 ± 39.9	1441.5 ± 31.1	304.4 ± 21.2	631.2 ± 13.8
MIP-1α	7.2 ± 7.8	182.2 ± 6.4	188.4 ± 18.4	460.1 ± 14.2	103.2 ± 3.9	288.7 ± 15.6
MIP-1β	73.6 ± 5.6	885.9 ± 122.3	248.7 ± 58.7	2318.5 ± 219.2	119.5 ± 47.7	1257.0 ± 64.4
RANTES	48.7 ± 22.9	164.1 ± 26.2	187.7 ± 70.0	239.2 ± 32.8	84.8 ± 5.7	107.2 ± 36.8
TNF-α	2.6 ± 6.3	26.4 ± 4.9	24.6 ± 8.5	43.7 ± 5.3	4.9 ± 0.4	34.0 ± 0.7

The cytokine and chemokine production of the cultured neutrophils with MPO-ANCA 24 h after stimulation was measured by the Bio-Plex® 23-plex cytokine detection system. The neutrophils were collected from C57BL/6J mice and were treated with MPO-ANCA plus CAWS mannoprotein as described in the Section 2 ($n = 4$ mice). The data are presented as the means ± SD of duplicate samples. n.d., not detected.

3.3. ANCA-induced IL-17 production is mediated by complement receptor-mediated pathways

Next, we investigated the effect of CAWS co-stimulation on the kinetics of MPO-ANCA-mediated IL-17A production. The mRNA transcription of *IL-17A* by neutrophils was observed 6–12 h after ANCA stimulation (Fig. 5a). In addition, this transcription was enhanced by CAWS co-stimulation. In contrast, CAWS has the ability to induce *IL-6* transcripts by peritoneal neutrophils alone 3–6 h after stimulation. Furthermore, *IL-6* mRNA transcription was also accelerated in the presence of MPO-ANCA whereas MPO-ANCA itself has no effect on transcription of *IL-6* by neutrophils. In addition, *IL-23p19* mRNA transcription by MPO-ANCA is also enhanced in the presence of CAWS. These results are consistent with the data obtained by the cytokine ELISAs. These result indicate that IL-6 expression in response to CAWS stimulation enhances the production of IL-17A and IL-23 by neutrophils, but IL-23 does not promote IL-17 production directly.

To better understand the downstream mechanism that mediates ANCA-induced IL-17A production, the effect of complement was analyzed, because the activation of those mediated pathways, especially the lectin pathway, are required for antifungal defense [29,30]. To support the hypothesis, CAWS has been demonstrated to promote complement-mediated activation [26]. In the presence of 1% complements, MPO-ANCA stimulation induced marked *IL-17A* transcription with or without CAWS, whereas *IL-23p19* transcription was significantly enhanced by only co-stimulation of CAWS (Fig. 5b). To further confirm the effect of the complement-mediated pathway, neutrophils were treated with pertussis toxin (PTX), which inhibits G-protein coupled receptor mediated signaling pathways (especially G_i) including the complement C5a receptor (CD88) [31–33]. Consistently, the *IL-17A* mRNA transcription of MPO-ANCA-stimulated neutrophils decreased in the presence of PTX (Fig. 5c). In contrast, neither complement nor PTX affects the *IL-23p19* transcription. These results suggest that the peritoneal neutrophils have the ability to produce IL-17 in response to MPO-ANCA via the complement-mediated signaling pathways. Next we speculated that $F(ab')_2$ fragment of MPO-ANCA affects less in IL-17 production if ANCA is dependent on complement pathway. We investigated whether MPO-ANCA induced IL-17 production also occurred by its $F(ab')_2$ fragment, we produced a $F(ab')_2$ fraction of MPO-ANCA ($F(ab')_2$-ANCA). $F(ab')_2$-ANCA partially promote CAWS-mediated IL-6 production (Fig. 5d). In addition, the result that IL-17A production was completely diminished by neutrophils, indicated that the neutrophil activation is mediated mainly via classical complement dependent pathways, which is initiated by the Fc region of antigen-captured antibody. Finally, we concluded that MPO-ANCA induces IL-6, IL-17A, and IL-23 production by activated neutrophils and promotes the conditions in which naïve Th cells differentiate into Th_{17} cells in concert with the production of TGF-β from inflammatory cells.

4. Discussion

We have demonstrated here that peritoneal neutrophils cause the initiation of systemic autoimmune inflammation by producing IL-17A and IL-23. IL-17 promotes the production of proinflammatory cytokines, including IL-1β, IL-6, IL-8 (in humans), and TNF-α by a subset of macrophages and epithelial cells [34]. A previous study demonstrated that airway neutrophils have an ability to produce IL-17 in LPS-induced lung inflammation [20]. We demonstrated here that peritoneal neutrophils also produce IL-17 in response to the autoantibody. IL-17 promotes Th_1 immune response to pathogenic fungi including *Aspergillus fumigatus*, *Cryptococcus neoformans*, and even *C. albicans* by IL-23 rather than IL-12 cytokine production [35–37]. In the model for systemic vasculitis, MPO-ANCA was injected in order to mimic a higher ANCA titer just as in human RPGN patients [14]. However, only administration of MPO-ANCA promotes higher levels of IL-1β, IL-12p40 and G-CSF production (Fig. 1a), implying an aberrantly higher ANCA titer than in conventional conditions is a trigger to induce proinflammatory cytokine production. In addition, the injection of CAWS alone failed to induce proinflammatory cytokine production even though activated macrophages infiltrated into pulmonary lesions (Fig. 1b). This suggests that a higher ANCA titer is a potential risk for a patient who has a hereditary factor for autoimmune disease onset.

Fungal infection and exposure to fungal-derived polysaccharides such as CAWS and β-glucans is also a potential risk for

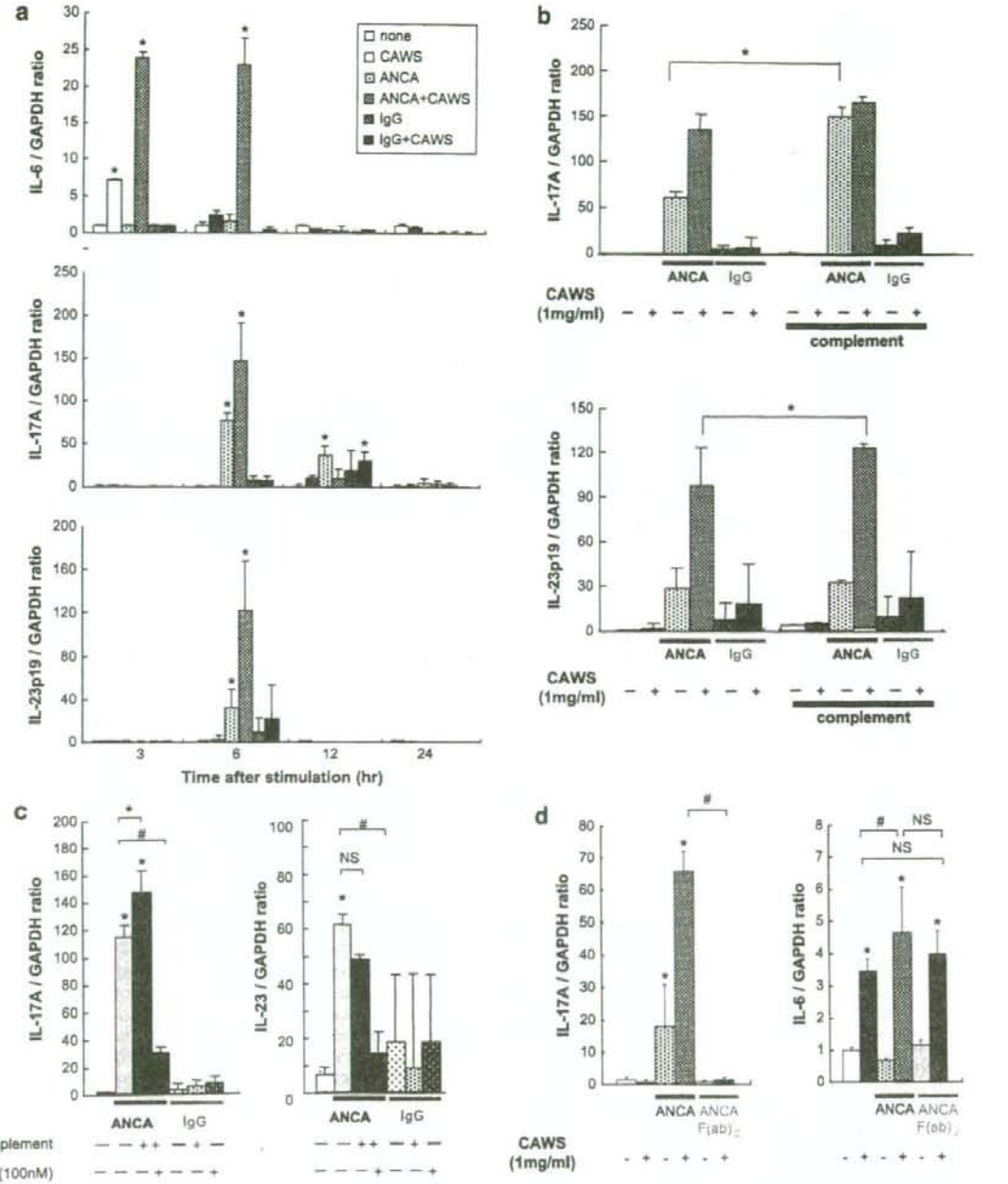

Fig. 5. Complement activation by MPO-ANCA is required for the ANCA-mediated IL-17 production by the activated neutrophils. (a) A time course of cytokine production profile. The mRNA transcription of *IL-6* (upper) *IL-17A* (middle) and *IL-23p19* (bottom) in peritoneal neutrophils with MPO-ANCA and CAWS. Freshly isolated peritoneal neutrophils by magnetic nanoparticles (1×10^6) stimulated with MPO-ANCA(1 mg/ml) or control IgG in the presence or absence of CAWS mannoprotein (1 mg/ml). The expression time course determined by quantitative RT-PCR are shown as the means ± SD of duplicate samples ($n = 3$). Asterisk (*), the differences from the transcription of the indicated column *vs* none were statistically significant ($p < 0.01$) according to Student's *t*-test. (b) IL-17 production by neutrophils were complement-dependent pathways. The mRNA transcription of *IL-17A* and *IL-23p19* in purified peritoneal neutrophils (1×10^6) with MPO-ANCA (1 mg/ml) in the presence or absence of 1% complement fragments. (c) Complement-mediated IL-17 production by neutrophils is regulated by a Gi-protein coupled receptor. The purified peritoneal neutrophils were stimulated with MPO-ANCA (1 mg/ml) or control IgG in the presence or absence of 1% mouse complement, and with or without 100 nM of pertussis toxin (PTX) pretreatment to block the Gi-mediated GPCR signaling pathway. The transcription of *IL-17A* and *IL-23p19* mRNA after 6 h stimulation determined by quantitative RT-PCR is shown as the mean ± SD of duplicate samples ($n = 3$). Astrisk (*), a significant difference between the two indicated columns ($p < 0.01$). (d) Fc-region of ANCA is involved in the ANCA-induced IL-17A production by neutrophils. The purified peritoneal neutrophils were stimulated with MPO-ANCA (1 mg/ml) or $F(ab')_2$ fragments of ANCA (1 mg/ml). The transcription of *IL-17A* and *IL6* mRNA after 6 h stimulation determined by quantitative RT-PCR is shown as the mean ± SD of duplicate samples ($n = 3$). A significant upregulation (*) or downregulation (#) between the two indicated columns ($p < 0.01$).

the initiation of an inappropriate autoimmune response. The receptor for CAWS is still unknown, but it is a complex of β-glucan and α-mannan [38]. Fungal β-glucan and α-mannan are recognized by the c-type lectin dectin-1 and dectin-2 respectively [39,40], which are expressed on macrophages, conventional DCs, and neutrophils. With the support of TLR2 [41–44], dectin-1 stimulates cDCs promote Th_{17} differentiation of $CD4^+$ T cells by IL-23p19 production via the Syk and CARD9 dependent pathways [45,46]. Neutrophils also have dectin-1 as a spliced variant form called dectin-1B [41], indicating that neutrophils can respond to fungal polysaccharides. These observations are consistent with our results that neutrophils also produce IL-23 and mediate the Th_{17}-medicated immune response. The higher ANCA titer is probably established by repeated MPO leakage on the membrane surface due to repeated or chronic fungal infection [28]. Th_{17} and IL-17-mediated immune responses are required for the appropriate immunity for the prevention of fungal infection, but unfortunately in some cases it facilitates the resting Th_{17}-mediated immune response while activating the neutrophils inappropriately via excessive proinflammatory cytokine induction, thus finally resulting in an autoimmune disorder.

IL-23 induces infiltration of neutrophils during acute infection and it is thought to induce development of Th_{17} cells through IL-17 production [18,27]. Strong IL-23 induction has been reported by some bacterial components, including peptidoglycans and some endogenous stimuli via G-protein-coupled receptors including prostaglandin E_2 [47–50]. In our model, IL-23 was not induced by CAWS solely but by the co-stimulation with MPO-ANCA stimulation. The fact that the co-stimulation of murine peritoneal neutrophils with MPO-ANCA, while CAWS also synergistically induced IL-23 production, suggests the ANCA-mediated neutrophil activation is facilitated by IL-23, which was reported to trigger systemic vasculitis via IL-17 and proinflammatory cytokines. Furthermore, CAWS have an ability to induce IL-6 by peritoneal neutrophils. *IL-6* mRNA transcription of peritoneal neutrophils is observed 3–6 h after stimulation, and co-stimulation of MPO-ANCA accelerates the transcription, subsequently resulting in enhanced IL-17A production after 12 h (see Fig. 5). MPO-ANCA affected less IL-6 production but promoted IL-23 production in concert with CAWS. Recent reports have described IL-6 to be a key cytokine for the differentiation of naïve Th cells to Th_{17} through the IL-21 autocrine loop and also for the upregulation of IL-23 receptors [51–53]. Therefore, ANCA-activated neutrophils facilitate the differentiation of naïve Th cells to Th_{17} cells by the quick combinatory production of IL-6, and following production of IL-17A and IL-23.

We revealed that least two separate pathways are required to mediate MPO-ANCA signaling and following cytokine production: via the Fc receptor mediated pathway and via MPO-ANCA-specific activation. The former can be explained by the theory that a large amount of IgG mediates pro- and anti-inflammatory activities through the engagement of the Fc fragments with its distinct Fcγ receptors (FcγR) [54,55]. Indeed, both MPO-ANCA and control IgG stimulation promote IL-17A production. However IgG-mediated IL-17A production was much less than that stimulated by ANCA. Moreover, MPO-ANCA stimulation expedited the IL-17A production in the presence of CAWS whereas the control IgG did not (Fig. 5a), thus implying that not great amounts of non-specific antibodies but autoantibodies are required for the neutrophil activation. In addition, our results show that the blockade of CD16/CD32 affects the cytokine production less (data not shown), indicating that ANCA signaling is mediated via other pathways except for their FcγR. To support this, the effect of ANCA signaling was ameliorated on addition of $F(ab)'_2$-ANCA (Fig. 5d). These results suggested that Fc-mediated activation of neutrophils is required for the ANCA-mediated immune response. This might be because the immune response of ANCA against MPO is mediated not by the FcγR on neutrophils but by the classical complement dependent pathways which require antigen-mediated aggregation of antibodies and following their Fc-region dependent activation of complements. The fact that additional complements enhanced the IL-17A production (Fig. 5c) indicated that the formation of the antigen–antibody complex triggered the complement activation of MPO-ANCA autoantibody. Thus, MPO-ANCA plays a significant role in the activation of neutrophils via the classical complement pathway.

The MPO expression on the membrane surface was assumed to activate the MPO-ANCA signaling, because MPO was enhanced by stimulation with proinflammatory cytokines [56]. The functional antigen–antibody complex of MPO and MPO-ANCA on the surface of neutrophils seems to induce the neutrophil activation and their secreted NETs [57]. Concurrently, the secondary stimulants to activate neutrophils were produced by the neutrophils themselves. The secondary stimulants are elevated humoral factors such as G-CSF and RANTES that were released in response to the innate defense and the reactive oxygen species produced by MPO and related peroxide enzymes released by neutrophils. Subsequently, neutrophils drove some adhesion molecules, including integrin molecules and some chemokines, which were induced by belatedly produced cytokines such as IL-6.

Our results have shown that ANCA-activated neutrophils produced IL-6, IL-17A and IL-23 and set up the conditions to amplify the Th_{17} response. TGF-β, which is an essential molecule for the differentiation of Th_{17} cells, is not produced by the activated neutrophils stimulated by MPO-ANCA nor CAWS. Both the TGF-β producing cells and the local environment of Th_{17} differentiation by MPO-ANCA therefore still need to be investigated.

In summary, this study revealed that the initial step of systemic vasculitis is due to the enhanced activation of neutrophils by fungal infection and MPO-ANCA in a murine experimental vasculitis model. Fungal infection and a high ANCA titer co-operatively trigger neutrophil activation by producing IL-6, IL-17 and IL-23, resulting in the provision of the local condition to promote IL-17 production and Th_{17} -mediated autoimmunity.

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症例報告

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血液透析導入時にヘパリン起因性血小板減少症をきたした関節リウマチの2症例

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key words：ヘパリン起因性血小板減少症，関節リウマチ，血液透析，メシル酸ナファモスタット，アミロイドーシス

〈要旨〉

血液透析の抗凝固薬としてヘパリンが広く用いられており，透析導入時にヘパリン起因性血小板減少症（heparin induced thrombocytopenia；HIT）を発生した報告は多くみられる．今回われわれは，関節リウマチ患者で，血液透析導入時にHITを発症した2症例を経験したため報告する．症例1は73歳，女性．慢性腎不全・関節リウマチにて通院中であった．大腿骨頸部骨折のため当院入院となったが，腎機能増悪のため術前に抗凝固薬にメシル酸ナファモスタットを用いて血液透析導入となった．股関節人工関節置換術施行後，抗凝固薬をヘパリンに変更したところ，血小板減少と体外循環回路内の凝血を認めた．抗凝固薬を再度メシル酸ナファモスタットに変更したところ，血小板数は速やかに改善した．症例2は70歳，女性．慢性関節リウマチにて近医通院していたが，腎機能増悪のため血液透析導入となった．透析導入後，血小板減少・肝機能障害・深部静脈血栓・四肢循環障害が出現し，全身状態悪化のため当院へ転院となった．抗凝固薬をヘパリンからメシル酸ナファモスタットに変更したところ，血小板減少・肝機能障害は改善したが，下肢壊疽部感染から敗血症となり永眠された．肝・腎・大腿動脈には血管炎はなく，血管壁へのアミロイドの沈着を認めた．両症例とも抗ヘパリンPF4複合体抗体が陽性であり，HITと診断した．関節リウマチは，HIT抗体を産生しやすい病態である可能性があり，透析導入の際にはHITの発症に注意する必要がある．

Two cases of heparin-induced thrombocytopenia in hemodialysis patients complicated by rheumatoid arthritis

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Although heparin is widely used as safe anticoagulant of hemodialysis therapy, heparin-induced thrombocytopenia (HIT) have been reported in hemodialysis initiation. We show here two cases of HIT patients complicated with rheumatoid arthritis. The first case：A 73 year-old woman, who had been consulting our hospital for both chronic renal failure and rheumatoid arthritis, was admitted due to femoral neck fracture. As renal dysfunction progressed after hospitalization, we treated her with hemodialysis therapy with nafamostat mesilate, an alternative anticoagulant used in Japan. One week after surgery, we changed the anticoagulant to unfractionated heparin. Thereafter, she developed thrombocytopenia and clot formation was observed in the dialyzer. When we switched the anticoagulant to nafamostat mesilate, her platelet count was gradually increased to the normal range. The second case was a 70-year-old woman, who had been consulting a local hospital for rheumatoid arthritis and chronic renal failure. Since her renal dysfunction became aggravated, she was admitted to another hospital and hemodialysis therapy was initiated with unfractionated heparin as the anticoagulant. One week after the start of

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hemodialysis, she suddenly developed thrombocytopenia, liver failure, deep vein thrombosis, and acrocyanosis with regional skin necrosis. After changing the anticoagulant from unfractionated heparin to nafamostat mesilate, thrombocytopenia and liver failure improved. However, severe sepsis developed due to the necrotic-infectious lesions involving the bilateral lower limbs. Necropsy specimens of her liver, kidney, and femoral artery demonstrated amyloid deposition along the vessel walls, but there was no vasculitis. We diagnosed both patients as having HIT type II based on the positive serum antibody against platelet factor 4-heparin complex on ELISA. Although the association of rheumatoid arthritis with HIT had rarely been reported, physicians might be careful regarding the development of HIT on hemodialysis initiation in patients with rheumatoid arthritis.

緒　言

ヘパリン起因性血小板減少症（heparin-induced thrombocytopenia；以下HIT）は，ヘパリン投与後に免疫学的機序により血小板減少・血栓形成などをきたす病態である．血液透析の抗凝固薬として未分画ヘパリンが広く用いられており，血液透析導入後のHIT発症はしばしば経験される[1]．一方，関節リウマチは，免疫学的異常をきたす疾患であり，ほかの自己免疫性疾患を合併することが知られている．今回われわれは，関節リウマチを背景疾患として有する患者で，血液透析導入時にHITを発症した2症例を経験したため報告する．

I．症　例

症例1：73歳，女性．

主訴：右大腿骨頸部骨折．

家族歴：特記すべきことなし．

既往歴：60歳より甲状腺機能低下症，70歳より関節リウマチ．

現病歴：腎硬化症による慢性腎不全にて3年前より当院外来通院していたが，腎機能は次第に増悪し，全身倦怠感・食欲低下出現．透析導入考慮中に転倒し，右大腿骨頸部骨折のため入院となった．

入院時現症：身長148.0 cm，体重49.5 kg，体温36.8℃，血圧198/70 mmHg，脈拍64/min．意識清明．皮疹なし．眼瞼結膜貧血あり．眼球結膜黄疸なし．呼吸音異常なし，収縮期駆出性雑音聴取．肝脾触知せず．右優位に下肢浮腫を認める．両手指PIP・MP関節変形あり．神経学的異常所見なし．

入院時検査所見（表1）：血液検査にて貧血を認めた．腎機能は，BUN 56.9 mg/dL，Cr 4.54 mg/dLであり，CCr 7 mL/minと低下していた．免疫学的検査では，リウマチ因子が115 IU/mLと高値を示していた．手指のX線では，PIP・DIP関節にびらん・関節の変形を認めた．股関節のX線で，右大腿骨頸部に骨折を認めた．

入院後経過（図1）：入院後，腎機能の悪化を認め，入院第3病日に血液透析導入（抗凝固薬はメシル酸ナファモスタット140 mg/4hr）．貧血に対し輸血を行った上で，第10病日に右股関節人工関節置換術を施行した．術後経過は良好であり，血液透析を継続しながらリハビリを開始した．しかし，血液透析での抗凝固薬をヘパリン（3,500 IU/4hr）に変更したところ，約10日後に血小板が7.4万/μLまで低下した．体外循環回路内の凝血も認めるようになったため，血液透析での抗凝固薬をメシル酸ナファモスタット（140 mg/4hr）に変更したところ，血小板数は速やかに改善し，回路内凝血もみられなくなった．経過良好なため，リハビリ目的にて他院転院となった．HITを疑い，血小板減少時に抗ヘパリンPF4複合体抗体を測定．後日陽性との結果を得て，HITと診断した．なお，転院後，抗凝固薬をヘパリンに変更したが，特に血栓・血小板の減少なども生じていない．

関節リウマチに関しては，症状が軽度であることや腎障害のため，過去に積極的治療は行っていなかった．今回入院中も関節症状は落ち着いており，特に治療は行わなかった．

症例2：70歳，女性．

主訴：右下腿浮腫，下腿壊疽，肝機能障害．

既往歴：50歳頃，関節リウマチ・甲状腺機能低下症．

家族歴：母：子宮癌，父：心不全．

習慣・嗜好歴：喫煙歴なし，飲酒なし．

現病歴：関節リウマチ・腎機能障害・甲状腺機能低下症にて近医通院されていた（プレドニゾロン5 mg/日，レボチロキシンナトリウム5 μg/日，内服中）．1年ほど前より，軽度腎機能障害を認めていた（Cr 1.62 mg/dL）．その後次第に，下腿浮腫，食欲低下出現し，腎機能も次第に増悪してきたため（Cr 3.30 mg/dL），近医入院となった．入院後より急激に呼吸困難出現し，肺うっ血を認めたため緊急血液透析導入となった（Cr 4.4 mg/dL，抗凝固薬ヘパリン）．しかしその約1

表 1　症例1 入院時検査結果

Urinalysis		Na	139 mEq/L
Pro	(2+)	K	4.3 mEq/L
OB	(−)	Cl	112 mEq/L
Glu	(±)	Serological test	
Sed.		CRP	0.65 mg/dL
RBC	1-4/HPF	IgG	1,385 mg/dL
WBC	30-49/HPF	IgA	527 mg/dL
hyaline cast	10-19/LPF	IgM	181 mg/dL
Peripheral blood		C3	53 mg/dL
WBC	4,700/μL	C4	10 mg/dL
Neut	62.8 %	CH50	38 U/mL
Lymph	25.8 %	Blood coagulation test	
Mono	3.8 %	PT	14.8 sec
Eo	7.3 %	APTT	33.6 sec
Baso	0.2 %	Hormonal examination	
Hb	6.7 g/dL	fT4	1.38 ng/dL
Plt	20.5×10^4/μL	TSH	1.69 μIU/mL
Blood chemistry		Immunological examination	
TP	4.9 mg/dL	RF	115 IU/mL
Alb	2.4 mg/dL	IgG-RF	(−)
GOT	20 IU/L	ANA	(−)
GPT	17 IU/L	PR3-ANCA	<10 EU
LDH	480 IU/L	MPO-ANCA	<10 EU
γ-GTP	43 IU/L	Anti-CLβ2GPI	(−)
T-Bil	0.8 mg/dL	LAC	(−)
BUN	56.9 mg/dL		
Cr	4.54 mg/dL		

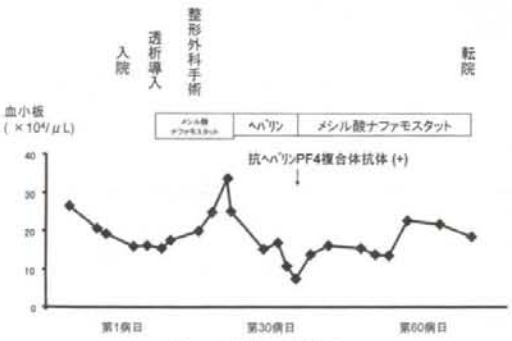

図 1　症例1 経過表

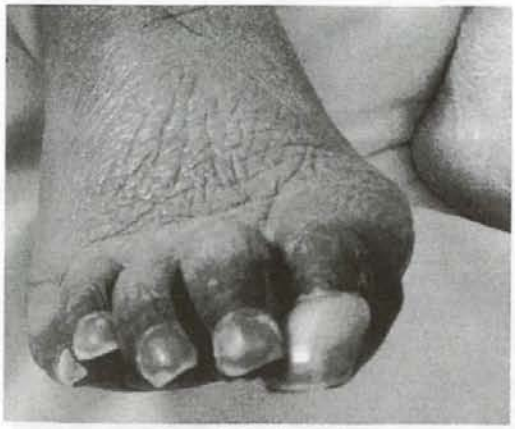

図 2　症例2 右足趾所見
右足趾の血流障害・壊疽を認める．

週間後に血小板数の減少（7万/μL），FDPの増加をきたした．DICを疑われ，ヘパリン，新鮮凍結血漿，アンチトロンビンⅢ製剤投与するも，さらに血小板数は減少．また，血小板減少と時期を同じくして，肝機能障害（GOT 2,611 IU/L，GPT 2,556 IU/L）・四肢循環障害（指先壊死・足趾壊疽）も出現．さらに，右大腿静脈に透析用ダブルルーメンカテーテルが挿入されていたが，右下腿浮腫増強あり，ドップラーエコーにて右大腿静脈の血栓・閉塞を認めた．前医での加療困難となり，当院に転院となった．

転院時現症：体温 36.1℃，血圧 136/92 mmHg，脈拍 98/分．意識清明．眼球結膜に黄疸，眼瞼結膜に貧血あり．甲状腺腫大あり．呼吸音異常なし．収縮期駆出性雑音聴取．肝脾触知せず．両下肢浮腫を認める．両足背動脈の触知良好．足趾は黒色炭化（図2）．両手指尖部にも血流障害を認める．

転院時検査所見（表2）：転院後の血液検査にて，血

表 2　症例 2 転院時検査結果

Peripheral blood		C3	65 mg/dL
WBC	3,500/μL	C4	9 mg/dL
Neut	42.5 %	CH50	28 U/mL
Lymph	37.6 %	Blood coagulation test	
Mono	14.5 %	PT	11.6 sec
Eo	4.6 %	APTT	36.4 sec
Baso	0.8 %	Fibrinogen	407 mg/dL
Hb	12.1 g/dL	FDP	39.9 μg/mL
Plt	2.9×10^4/μL	TAT	12.0 ng/mL
Blood chemistry		D-dimer	21.9 μg/mL
TP	5.5 g/dL	AT Ⅲ	42 %
Alb	2.3 g/dL	Viral infection	
GOT	131 IU/L	HA-IgM-Ab	(−)
GPT	137 IU/L	HBs-Ag	(−)
LDH	660 IU/L	HCV-Ab	(−)
ALP	526 IU/L	Hormonal examination	
γ-GTP	151 IU/L	fT4	1.24 ng/dL
T-Bil	5.8 mg/dL	TSH	0.29 μIU/mL
BS	108 mg/dL	Immunological examination	
BUN	51.1 mg/dL	RF	249 IU/mL
Cr	3.75 mg/dL	IgG-RF	(−)
Na	137 mEq/L	ANA	(−)
K	3.8 mEq/L	PR3-ANCA	<10 EU
Cl	105 mEq/L	MPO-ANCA	<10 EU
CK	52 IU/L	Cryoglobulin	(−)
Serological test		IC (C1q)	(−)
CRP	7.44 mg/dL	Anti-dsDNA-IgG	(−)
IgG	1,621 mg/dL	Anti-CLβ2GPI	(−)
IgA	503 mg/dL	LAC	(−)
IgM	112 mg/dL	PAIgG	198.0 ng/10^3Plt
		Anti-Mi	(−)

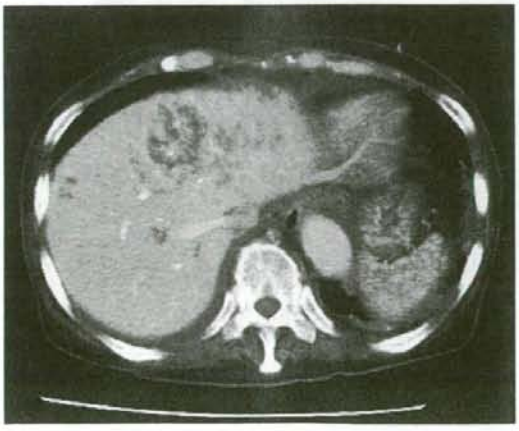

図 3　症例 2 腹部造影 CT
肝臓に多発性の低吸収域を認める．

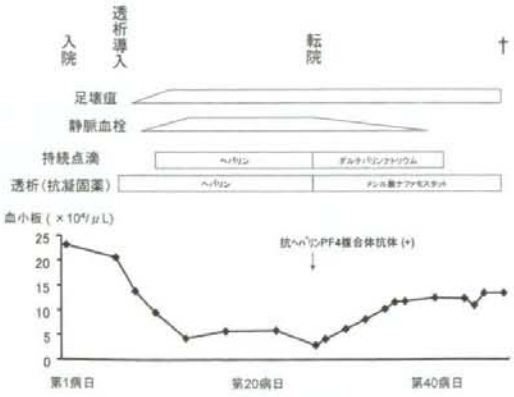

図 4　症例 2 経過表

小板数は 2.9 万/μL と著明に低下していた．また，GOT 131 IU/L，GPT 137 IU/L，ALP 526 IU/L，γ-GTP 151 IU/L と，前医よりも改善傾向ではあったが，肝機能障害を認めた．CRP 7.44 mg/dL と炎症所見を認めた．FDP 39.9 μg/mL，D ダイマー 21.9 μg/mL と凝固線溶系の亢進を認めた．リウマチ因子は 249 IU/mL と上昇していたが，IgG 型 RF は陰性であった．ほか自己抗体などは陰性であった．

画像検査では，腹部造影CTにて肝臓に多発性の低吸収域を認めた（図3）．サーモグラフィにて両手・両足末梢で著明な低温像を認めた．下肢エコーでは，右大腿静脈から膝下静脈にかけて血栓を認めた．

転院後経過（図4）：転院後も3回/週の血液透析を継続した．透析の抗凝固薬は，出血傾向があることも考慮し，メシル酸ナファモスタット（140 mg/4hr）を用いた．また，深部静脈の血栓に対し前医から継続してヘパリンの持続投与を行っていたが，血小板数の著明な低下に伴い出血傾向もみられたため，ダルテパリンナトリウムの持続点滴（5,000 IU/日）に変更した．経過とともに血小板数・FDP・Dダイマーなどの所見は改善した．

肝障害に関しても，経過とともに改善を認めた．関節リウマチに対しては，関節変形はあるものの，関節腫張・疼痛は軽度であり，前医にて処方されていたステロイド（プレドニゾロン5 mg/日）を継続した．四肢循環障害に関しては，プロスタグランジン製剤を投与しながら，皮膚科的処置を継続した．

しかし全身状態は次第に増悪し，当院転院約1か月後に敗血症のため永眠された．死後，家族の同意を得てネクロプシーを施行した．肝は，非特異的な炎症細胞浸潤を軽度認め，動脈血管壁にアミロイド沈着を認めた．腎にもアミロイドの沈着を認めた（AAアミロイドーシス）．大腿動脈に血管炎の所見はなく，動脈硬化性変化も認めなかった．右足皮膚は，皮下脂肪組織に炎症細胞浸潤を軽度認めるのみであった．

入院時血小板減少が著明であったことと，四肢の血栓を認めたことより，HITを疑い，抗ヘパリンPF4複合体抗体を測定し，後日陽性との結果を得た．ネクロプシーの結果もあわせ，関節リウマチ・アミロイド血管症を背景に，ヘパリン使用によりHITを発症したため，血小板減少・血栓による四肢末梢の循環障害・静脈血栓症などをきたしたものと考えられた．肝障害に関しては，ネクロプシーのため病変部位がとれていない可能性があり，正確な診断は困難であった．しかしながら，画像所見なども併せて，アミロイド血管症を背景として，HITによる血栓症が合併して生じた病変であると推察された．なお，リウマチ性血管炎・コレステロール塞栓症などは，ネクロプシーの結果より否定的であった．

Ⅱ．考　察

HITは，非免疫機序で発生するⅠ型と，ヘパリン依存性の自己抗体が出現するⅡ型に分類される．本二症例でみられたⅡ型は，血小板第4因子（PF4）とヘパリンの複合体を抗原とする自己抗体（以下HIT抗体）が産生されることによりおこる．HIT抗体は，血小板や血管内皮細胞の活性化をひきおこすことにより，血小板機能亢進および凝固亢進状態を生じ，動静脈に血栓症をひきおこす．症例1では，血小板減少に加え体外循環回路内の凝血がみられた．症例2では，血小板減少に加え急激な動静脈血栓を発症し，激烈な経過をたどった．両症例とも関節リウマチを基礎疾患として有しており，関節リウマチに伴う免疫学的異常やアミロイドーシスなどが，HITの発症とその経過に影響を及ぼした可能性が考えられる．

関節リウマチとHITの合併報告は少ない[2,3]．しかしほかの自己免疫性疾患におけるHITの発症報告は散見される[4–6]．関節リウマチはほかの自己免疫性疾患を合併することがよく知られており，特発性血小板減少性紫斑病などの合併報告例もみられる[7,8]．さらに，関節リウマチ患者では，末梢血中の血小板は活性化していることが知られており[9]，血漿PF4が上昇しているとの報告もある[10]．本二症例において関節リウマチとHITが合併した原因は不明であるが，上述のことをあわせて考えると，関節リウマチはヘパリン投与によりHIT抗体を産生しやすい病態である可能性が考えられる．また，本二症例の関節リウマチの特徴としては，活動性が低く，治療としては症例2に少量のステロイドが投与されていたのみであった．このことも，本二症例のHIT抗体産生に影響した可能性がある．

さらに，関節リウマチは，アミロイドーシス・薬剤・ほかの膠原病の合併などで腎機能障害をきたしやすく，透析導入となる症例もまれではない[11]．今後，関節リウマチ合併の慢性腎不全患者に対し，透析導入時にヘパリンを使用する際は，HITの発症に注意する必要性が示唆された．

透析患者のHIT発症は透析導入期に集中しており，維持透析期には少ないといわれている[1,12]．またHITは女性に多いとの報告もある[13]．本二症例は，いずれも透析導入期の発症で，かつ女性であったことから，よりハイリスク群であったと考えられる．

HITの病因であるHIT抗体の検出には，C^{14}標識セロトニン法・血小板凝集法・フローサイトメトリー法・酵素免疫測定法（ELISA）などの方法が用いられる．本邦で行われているのは主にELISA法であり，本症例もこの方法で測定した（検査機関はSRL，ELISAキットはDiagnostica Stagoを使用）．このELISA法は，血小板を活性化しない抗体を測定している可能性

もあるので，ヘパリン投与後の血小板減少などの臨床経過も含めて，HITと診断することになる．HIT抗体は，検査施設によっては結果を得るまでに時間を要する．特に症例2のように鑑別診断が多い複雑な病態では迅速な診断が要求されるため，HIT情報センターなどの専門機関への測定依頼や[14]，より簡便なHIT抗体の測定法の開発が望まれる．

症例2では，透析での抗凝固薬として，ヘパリンを中止しメシル酸ナファモスタットを使用することにより，血小板数や血栓傾向を反映するDダイマーなども改善した．過去にもHITにメシル酸ナファモスタットが有効であったとの報告もある[15~17]．確かに，メシル酸ナファモスタットはヘパリンの中止という点では有効である．しかし，HIT発症時の過凝固状態においては，抗トロンビン活性の弱いメシル酸ナファモスタットのみでは不十分であると考えられている．そのため，現在のところ本邦ではHITに対し（平成19年12月現在，保険適応外ではあるが）抗トロンビン作用の強いアルガトロバンを使用することが推奨されている[18]．本二症例ではメシル酸ナファモスタットを使用し，血小板減少や血栓傾向は改善したが，メシル酸ナファモスタット単独での代替使用には注意が必要である．なお，本邦におけるHITの情報は，HIT情報センターのホームページ（http://www.hit-center.jp）が参考となる．

低分子ヘパリンは未分画ヘパリンと交差反応を示すため，HITを増悪させる可能性がある．そのため，HITが強く疑われる患者には，血栓症の合併の有無にかかわらず，低分子ヘパリンは投与しないこととされている．さらに，低分子ヘパリンでも透析導入時にHITを生じたとの報告もある[19]．症例2は当院転院後，深部静脈血栓に対して，未分画ヘパリンから低分子ヘパリンに変更したが，結果として病態を悪化させた可能性が否定できず，反省すべき点であった．

結　語

関節リウマチ患者に，透析導入後ヘパリン起因性血小板減少症を合併した二症例を経験した．関節リウマチ患者は，しばしば腎機能障害をきたし血液透析導入となるが，血液透析導入時に血小板減少・血栓・回路内凝固などをきたした際は，HITを考慮し速やかに抗凝固薬の変更をする必要があると考えられた．

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ORIGINAL ARTICLE

Renal pathology of ANCA-related vasculitis: proposal for standardization of pathological diagnosis in Japan

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Abstract
Background In Japan, systematic evaluation of the histologic parameters of anti-neutrophil cytoplasmic autoantibodies (ANCA)-related vasculitis has been performed according to the Japanese classification by Shigematsu et al. However, this classification is quite different from that of the European Vasculitis Study Group (EUVAS) classification. Therefore, a histological common basis is needed to compare Japanese histological data with the international database.
Method Histological parameters concerning glomerular, tubulointerstitial, and vascular lesions of ANCA-related

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vasculitis, which are indispensable for clinical management, were elucidated and defined by reviewing, utilizing the merits of, and amending the two scoring systems.
Results and conclusion A new comprehensive and standardized scoring system, by which histological quantitative assessment can provide evidence for therapy planning, has been developed for renal biopsy of Japanese ANCA-related vasculitis.

Keywords Renal biopsy · Glomerulonephritis · Microscopic polyangiitis · Wegener's granulomatosis · Renal limited vasculitis · ANCA-related vasculitis · Histopathological evaluation

Introduction

Anti-neutrophil cytoplasmic autoantibody (ANCA)-related systemic vasculitis is an important disease of the elderly, and it is increasingly recognized as a life-threatening disease [1]. Since the kidney is highly vascularized, vasculitis frequently occurs in the kidney. The histopathological findings of ANCA-related vasculitis in the kidney are considered to show a variety of lesions, of which crescentic and/or focal necrotizing glomerulonephritis as well as small vessel arteritis are the most prominent [2]. Early recognition of the disease facilitates prompt treatment and results in better prognosis; however, the use of basic laboratory indicators, such as hematuria, proteinuria, or the serum creatinine level, is considerably limited in facilitating the prediction of the affected site of vasculitis [1, 3]. From this point of view, histologic examination of renal tissue may be clearly diagnostic and provide information, if inflammation and necrosis can be seen in various sites of vessels, including arteries, arterioles, and glomerular capillaries. In particular, distinguishing the acute phases of vasculitis from the chronic phase is a critical step in the characterization of the disease, and an appropriate evaluation is required to characterize the chronicity of the disease in order to guide treatment decisions and to evaluate the response to treatment. Indeed, in the setting of chronic disease, immunosuppressive therapy is less effective and may actually worsen the outcome by predisposing patients to opportunistic infections [4]. Renal biopsy can help distinguish treatment-responsive active disease states from treatment-unresponsive chronic disease states.

Currently in Japan, the choice of therapy, which may influence the outcome of this disease, varies among physicians. This may be because reproducible histologic parameters have not been established to identify the morphological conditions as in the forthcoming multi-center study to establish standardized therapy. This first requires evidence, from which standardized qualitative and quantitative histologic assessment can provide the clinical basis for treatment decisions. Treatment response as well as treatment populations among several clinical studies can then be compared on the basis of histological evaluation. The information obtained in the follow-up biopsy may also form the basis of an individual patient's evaluation for long-term care and may additionally be helpful in determining the prognosis or outcome. Treatment regimens used for induction and maintenance should therefore be based on clinical as well as pathological features [4–6].

The recent European multi-center controlled trials, coordinated by the European Vasculitis Study Group (EUVAS), have provided definitive evidence for the treatment of patients with ANCA-related vasculitis, on which common quantitative pathological parameters are based [7–14]. In Japan, few proposals for evaluating histology have been reported from the aspect of the above clinical use. Shigematsu et al., as members of the group working on "Progressive Renal Diseases from the Specially Selected Diseases of the Ministry of Health and Welfare Research Project" (1996–1998), developed a histological scoring system in an effort to produce guidelines for the effective treatment of rapidly progressive nephritic syndrome (WHO) [15, 16]. However, this classification is quite different from the EUVAS classification, and it is problematic to compare Japanese histological data with the international database. Therefore, there is a clear need for the comprehensive and standardized assessment of the histology of this disease for the Japanese population with ANCA-related vasculitis.

In this review, in reference to the EUVAS histological scoring system and Shigematsu's histological grading and staging system, histological parameters, which are indispensable for clinical management, are elucidated, and a standardized scoring system for renal biopsy is proposed for Japanese ANCA-related vasculitis.

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Histological parameters

Glomeruli

Wegener granulomatosis (WG), microscopic polyangiitis (MPA), and Churg-Strauss syndrome (CSS) share

pathologically identical glomerular lesions such as pauci-immune necrotizing and/or crescentic glomerulonephritis; however, varied morphology is involved according to chronological development from the early to late stage of glomerular lesions. For example, endocapillary lesions, glomerular tuft necrosis, and cellular and fibrocellular crescents represent acute and active lesions, whereas fibrous crescent, adhesion/synechia, and global or segmental glomerulosclerosis are chronic lesions. Because the clinical profiles of these vasculitic syndromes overlap, the diagnosis may sometimes be uncertain.

Endocapillary lesions

At a very early stage of ANCA-related glomerulonephritis, neutrophilic infiltration into the glomerular capillary lumen can be seen, which is followed by a microthrombosis before disruption of the glomerular capillary wall (Fig. 1a, b). In electron microscopy, neutrophils show degranulation together with marked subendothelial edema due to endothelial injury. This electron-microscopic finding is a characteristic feature of ANCA-related glomerulonephritis (Fig. 2).

Glomerular tuft necrosis

Destruction of the glomerular basement membrane (GBM) and/or mesangial matrix is designated as glomerular tuft necrosis, which reflects focal segmental necrotizing glomerulonephritis. Fibrin or fibrinoid material is also often present inside as well as outside of the GBM (Fig. 3). This lesion can be seen with or without crescent formation and is often present when a patient presents with microhematuria without apparent severe proteinuria and without elevation of the serum creatinine level.

Crescent (cellular, fibrocellular, fibrous)

Crescent is defined as the extracapillary cellular proliferation of more than two cell layers occupying one-fourth or more of the glomerular capsular circumference, excluding podocytic hyperplasia [17]. Localized extracapillary hypercellularity (so-called small crescent) is defined as a lesion of less than 25% of the glomerular capsular circumference with extracapillary hypercellularity of more than two cell layers thick [7]. By a combination of cells and extracellular matrix, the crescent is divided into cellular, fibrocellular, and fibrous crescent. Cellular crescent is defined when more than 50% of the crescent is occupied by the cells (Fig. 4a). Fibrous crescent is defined when more than 90% of the crescent is occupied by extracellular matrix (Fig. 4b); therefore, fibrocellular crescent is defined when less than 50% of the crescent is occupied by cells and less than 90% of the crescent is occupied by extracellular matrix (Fig. 4c) [7, referred from a Consensus Meeting on the Clinico-pathological Classification of IgA nephropathy in Oxford, 2005]. Circumferential and segmental crescents are defined as crescentic lesions occupying more than 50% and within 50% of Bowman's space, respectively [7]. Cellular crescent may progress to fibrocellular crescents, which are composed of epithelial cells and fibrous materials, and are often seen together with disruption of the Bowman's capsular basement membrane.

Mesangial cell proliferation

Mesangial cell proliferation is defined as a glomerulus with more than three nuclei per peripheral mesangial area that is not affected by sclerosis or extracapillary proliferation [7, 18]. This parameter is an uncommon lesion caused by pauci-immune necrotizing and/or crescentic glomerulonephritis.

Destruction of the basement membrane of Bowman's capsule

Together with marked crescent formation, destruction of the basement membrane of Bowman's capsule is typically present in the context of ANCA-related vasculitis. The lesion is often surrounded by infiltrating macrophages, lymphocytic cells, and plasma cells (Fig. 5).

Periglomerular dense inflammatory infiltrates (with or without the destruction of Bowman's capsule) is similar to that seen with global glomerulosclerosis (Fig. 6). A granulomatous reaction is defined as an accumulation of epithelioid cells with or without giant cells around the disrupted Bowman's capsule (Fig. 7). Fibrotic lesions with fibroblast and fibrous materials, filling Bowman's space, are often present in the context of rupture and splitting of the Bowman's capsular basement membrane. Inflammatory cell types may include macrophages, lymphocytes, plasma cells, and neutrophils.

Adhesion/synechia

An adhesion/synechia is defined as a lesion with a local (less than 25% of the circumference of Bowman's capsule) area of fibrous continuity between the glomerular tuft and Bowman's capsule (Fig. 8) [7, 18]. Further, a small adhesion of the flocculus with Bowman's capsule does not show extracapillary proliferation, thereby differentiating it from a fibrous crescent.

Global or segmental glomerulosclerosis

Global sclerosis is defined as a sclerotic area with scarring involving more than 50% of the glomerular tuft. This

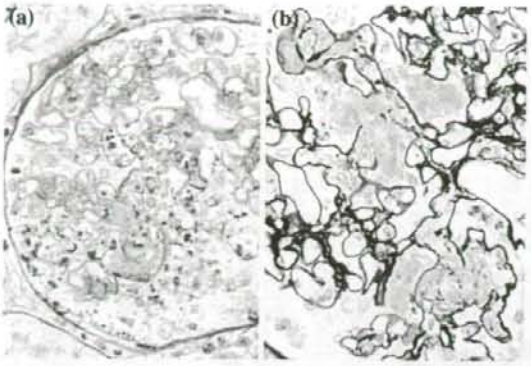

Fig. 1 Early stage of MPO-ANCA-related glomerulonephritis. Neutrophilic infiltration into the glomerular capillary lumen can be seen (**a**), followed by microthrombosis before disruption of the glomerular capillary wall (**b**). (**a** PAS stain, (**b**) PAM stain)

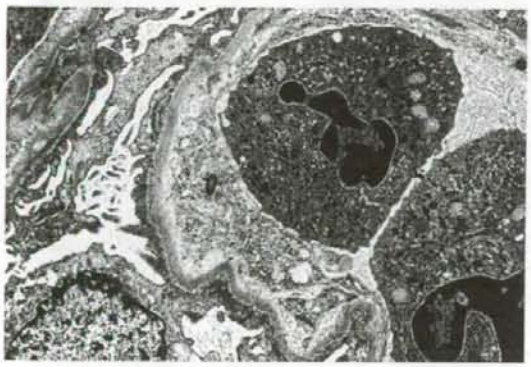

Fig. 2 Electron-microscopy findings of MPO-ANCA-related glomerulonephritis. Degranulated neutrophils are present with marked subendothelial edema due to endothelial injury. (Double-stained with uranyl acetate and lead citrate. ×10,000)

lesion is present at the end stage of crescentic glomerulonephritis, from which ischemic or obsolescent glomeruli should be excluded (Fig. 9). Segmental sclerosis is defined as a sclerosed area of scarring involving less than 50% of the glomerulus [17].

Tubulointerstitium

ANCA-related vasculitis sometimes involves interstitial inflammation, which is known as peritubular capillaritis. This lesion is defined by the presence of inflammatory cells within the capillary lumina, which often adhere to the capillary wall. Inflammatory cells consist mainly of neutrophils mingled with lymphocytes and plasma cells

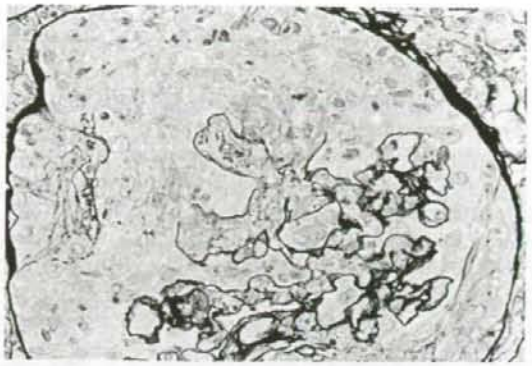

Fig. 3 Glomerular tuft necrosis. Destruction of the glomerular basement membrane induces cellular crescent formation in which fibrin is present inside and outside of the GBM (PAM stain)

(Fig. 10). Even when glomerular lesions are not remarkable, prominent tubulointerstitial inflammation can be seen in association with an impairment of renal function. Tubulointerstitial lesions are composed of interstitial inflammation, tubulitis, destruction of the tubular basement membrane (TBM), peritubular capillaritis, phlebitis, and granulomatous lesion. These lesions present as an acute and active process, whereas interstitial fibrosis and tubular atrophy are chronic lesions.

Interstitial inflammation

Interstitial inflammation is defined as an excess of inflammatory cells within the cortical interstitium, excluding the subcapsular area and the area of surrounding global glomerulosclerosis [7]. Accumulation of polymorphs as well as mononuclear cells in the peritubular capillary associated with the disruption of the capillary wall is defined as peritubular capillaritis, which is often combined with extravasation of erythrocytes [19].

Tubulitis and destruction of the TBM

According to the Banff 97 classification [19], grades of tubulitis ("t" score) are based on the greatest number of infiltrating mononuclear cells in the tubular epithelium (that is, having breached the TBM and lying beneath or between tubular cells) per tubular cross-section; if the tubule is sectioned longitudinally, the results are expressed per ten tubular cells (the average number of cells per cross-section). EUVAS scoring uses the term "intra-epithelial infiltrate" instead of "tubulitis" [7]. Inflammatory tubular injury, destruction of tubular basement membranes, and/or acute tubular necrosis is included as significant histologic findings in the "t3" grade [19]. In the case of ANCA-

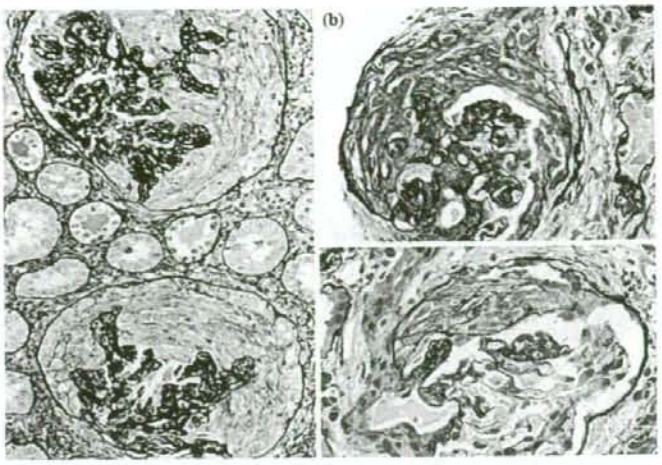

Fig. 4 Glomerular crescent. Cellular crescent = more than 50% of the crescent occupied by cells (**a**) (PAM stain). Fibrous crescent = more than 90% of the crescent occupied by extracellular matrix (**b**) (PAM-Masson stain). Fibrocellular crescent = less than 50% of the crescent occupied by the cells and less than 90% of the crescent occupied by extracellular matrix (**c**) (PAM-Masson stain)

Fig. 5 Destruction of the basement membrane of Bowman's capsule. Infiltrating lymphocytic cells, plasma cells, and macrophages surround the lesion (PAM stain)

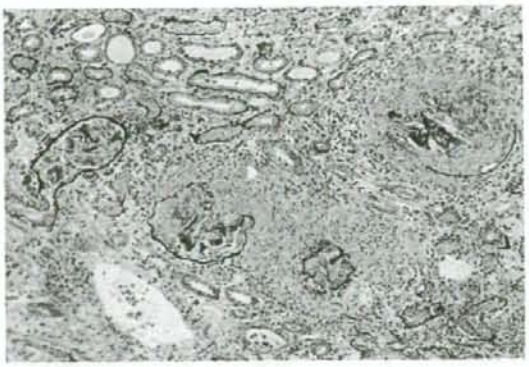

Fig. 7 Granulomatous reaction with destruction of Bowman's capsule. Accumulation of epitheloid cells is seen around a disrupted Bowman's capsule and sclerotic glomerulus (PAM-Masson stain)

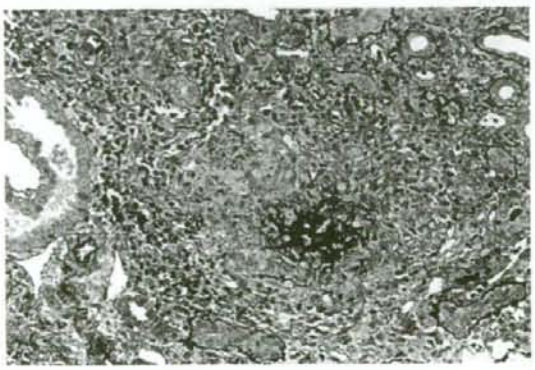

Fig. 6 Destruction of Bowman's capsule around a sclerotic glomerulus. Periglomerular granulomatous dense infiltrate is seen around a sclerotic glomerulus with destruction of Bowman's capsule (PAM stain)

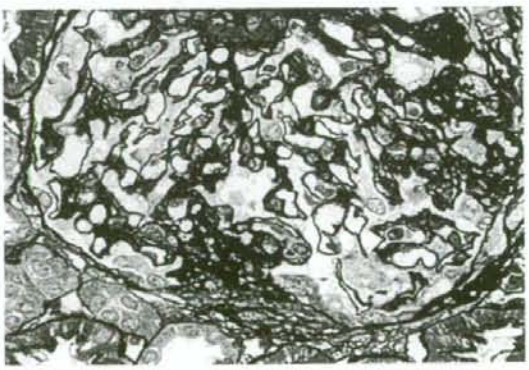

Fig. 8 Adhesion/synechia. A lesion with a local area of fibrous continuity between the glomerular tuft and Bowman's capsule is seen without extracapillary proliferation (PAM stain)

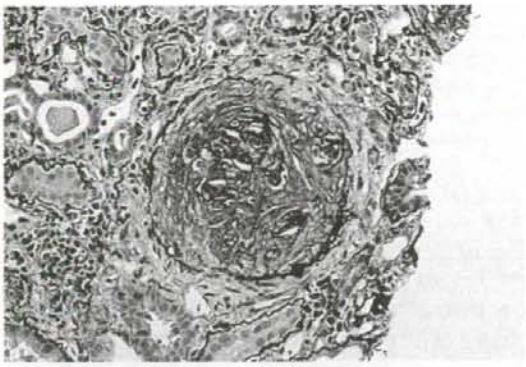

Fig. 9 Global sclerosis of the glomerulus of ANCA-related vasculitis. Bowman's space is occupied by a proliferation of collagen fiber mingled with epithelial cells. The basement membrane of Bowman's capsule is disrupted, inducing a periglomerular fibrosis (PAM-Masson stain)

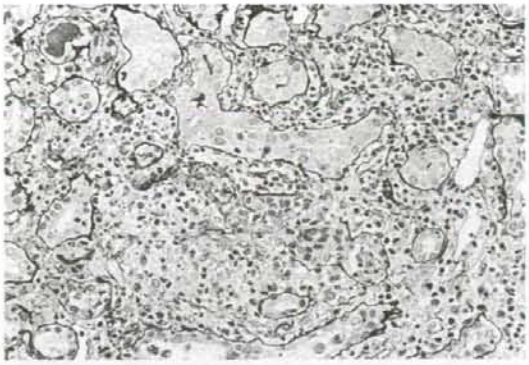

Fig. 11 Disruption of the tubular basement membrane induces a granulomatous reaction in MPO-ANCA-related interstitial inflammation (PAM stain)

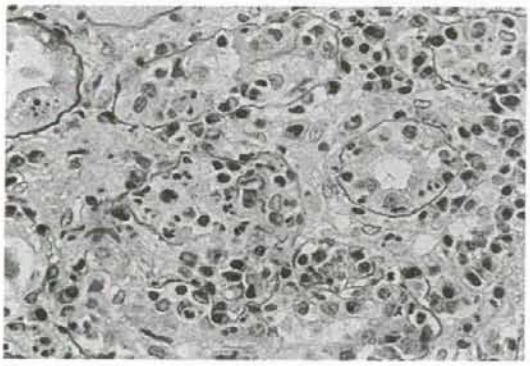

Fig. 10 ANCA-related tubulointerstitial nephritis. Early phase of ANCA-related peritubular capillaritis may involve a tubulointerstitial lesion, which consists of neutrophils mingled with lymphocytes and plasma cells (PAS stain)

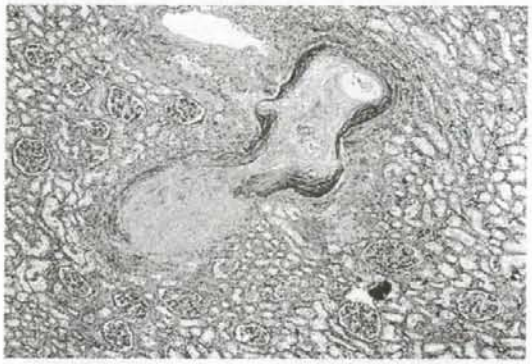

Fig. 12 MPO-ANCA-positive polyarteritis nodosa. Arcuate artery shows necrotizing arteritis without glomerular involvement (PAM stain)

related interstitial inflammation in Japan, almost all of the cases can be graded as "t3" because destruction of the TBM is usually present (Fig. 11). An interstitial granulomatous lesion can be seen together with destruction of the basement membrane of Bowman's capsule or TBM (Figs. 6, 7, 11).

Interstitial fibrosis and tubular atrophy

Tubular atrophy is defined by a thick irregular TBM with decreased diameter of the tubules. It is scored according to the percent of the cortical area involved, excluding the subcapsular area. Interstitial fibrosis is defined as increased

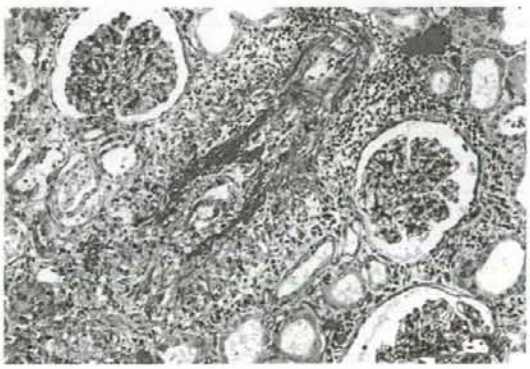

Fig. 13 Fibrinoid necrosis in a small artery in an MPO-ANCA-positive patient (Masson stain)