# Leukocytes and the kidney contribute to interstitial inflammation in lupus nephritis

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**Interstitial leucocyte infiltration, a prominent feature of lupus nephritis, predicts deterioration of renal function. We used two models of lupus nephritis in mice, one with chronic spontaneous disease and the other with acute interferon-α (IFNα)-mediated disease. The latter is characterized by the virtual absence of interstitial infiltration. *In vivo* migration assays showed that splenic leukocytes from spontaneously nephritic mice tended to migrate into non-inflamed syngeneic kidneys. This was enhanced if the recipient kidneys were already inflamed. Kidneys from both chronically and acutely nephritic mice showed similar ability to recruit splenic leukocytes from chronically diseased mice. Leukocytes from acutely diseased mice, however, failed to migrate into chronically inflamed kidney. Compared with those with chronic nephritis, the kidneys of acute nephritic mice expressed less of the inflammatory chemokine CXCL13/BLC. Moreover, leukocytes from acute nephritic mice displayed impaired migration, *in vitro*, to T-cell chemokine attractants. This study links leukocyte infiltration to both kidney chemokine expression, and leukocyte chemotaxis to kidney-expressed chemokines.**

*Kidney International* (2008) **73**, 172–180; doi:10.1038/sj.ki.5002625; published online 10 October 2007

KEYWORDS: lupus nephritis; interstitial inflammation; leukocyte recruitment; interferon alpha; chemokines

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**Received 17 October 2006; revised 16 July 2007; accepted 7 August 2007; published online 10 October 2007**

Lupus nephritis represents a major cause of morbidity and mortality in systemic lupus erythematosus (SLE), and is characterized by extreme diversity of pathological manifestations. Histologically, a spectrum of glomerular lesions occurs, ranging from no or mild mesangial proliferation to full-blown proliferative and crescentic glomerulonephritis.[1] Along with glomerular lesions, inflammatory infiltrates are frequently observed in the interstitium of SLE patient kidneys.[2,3] The severity of interstitial inflammation correlates with the degree of renal damage,[4,5] and it predicts progression to renal insufficiency.[6–9] Inflammatory interstitial infiltrates in SLE nephritis are predominantly composed of T and B lymphocytes and macrophages.[2,10–12] They may contribute through cytokine/chemokine release to the kidney lesions observed in SLE.[13] In animal models of lupus that develop a disease close to human SLE and die of end-stage renal failure,[14] lupus nephritis is characterized, as in human SLE, by variable degrees of glomerular and tubulointerstitial inflammation comprised of mononuclear cells (ie, T, B, lymphocytes, plasma cells, and macrophages).[15–17]

The mechanisms that underlie the accumulation of leukocytes in the interstitium of the kidney of SLE patients or of lupus mice remain to be defined. In particular, whether leukocytes and/or kidney have particular properties that favor such migration is an important question for a better understanding of lupus nephritis pathogeny. To address this issue, we compared renal alterations and leukocyte migration to the kidney in a chronic model of lupus nephritis (ie, unmanipulated NZB × NZWF1 (NZB/W) mice) and in an acute model of disease (ie, young NZB/W mice treated *in vivo* with interferon-α (IFNα)).[18,19] Our data show that acute nephritis differs from chronic nephritis by the virtual absence of interstitial leukocyte infiltration. Adoptive transfer experiments and characterization of a series of chemokines at the kidney and leukocyte levels reveal differences in both renal chemokine expression and chemotaxis of spleen leukocytes to kidney-expressed chemokines between the two models of lupus nephritis. Thus, leukocyte interstitial infiltration in lupus nephritis (or its absence) is linked to coordinated action of both kidney chemokine expression and leukocyte chemotaxis to kidney-expressed chemokines.

## RESULTS

### Mechanism of leukocyte recruitment to the kidneys in lupus nephritis

Renal histological examination of spontaneously nephritic NZB/W mice showed a severe diffuse glomerulonephritis with either segmental or global involvement of all glomeruli (Figure 1c and d). Importantly, large perivascular interstitial infiltrates were present (Figure 2, left panel). On immunohistochemical assessment, these inflammatory infiltrates (Figure 3a and b) were found to be composed of ~60% of B cells (Figure 3d and e), ~30% of T cells (Figure 3g and h), and of <10% of myeloid cells (Figure 3j and k).

To test whether accumulation of leukocytes in kidneys from mice with chronic lupus nephritis was due to active recruitment from the circulation, we performed transfer experiments with fluorescently labeled spleen leukocytes. Leukocytes from spontaneously nephritic NZB/W donors were labeled and transferred into nephritic NZB/W recipients. Parallel experiments were performed with Balb/c mice. The numbers of labeled cells detected in the kidneys of NZB/W recipient mice were significantly higher than those detected in the kidneys of Balb/c mice (Table 1). This increased recruitment involved both B (CD19+) and T (CD3+) lymphocytes as well as myeloid (Mac1+) cells.

### Role of leukocytes vs kidney in leukocyte recruitment

To determine the respective contribution of leukocytes vs inflamed kidneys in the increased recruitment of circulating leukocytes, we injected fluorescently labeled spleen leukocytes from young or spontaneously nephritic NZB/W to either young or diseased NZB/W mice. Parallel experiments were performed with Balb/c mice. In all combinations tested, leukocyte recruitment was minimal in Balb/c mice (Figure 4a, c, e). When leukocytes from spontaneously nephritic NZB/W were injected to young NZB/W mice, cell recruitment was slightly but not significantly increased as compared with controls (ie, 'young' donor cells to 'young' recipient kidney). Conversely, migration of leukocytes from young NZB/W to kidneys of spontaneously nephritic NZB/W mice was moderately but not significantly increased as compared with controls. Strikingly, leukocytes from diseased NZB/W showed significant migration into the kidneys of

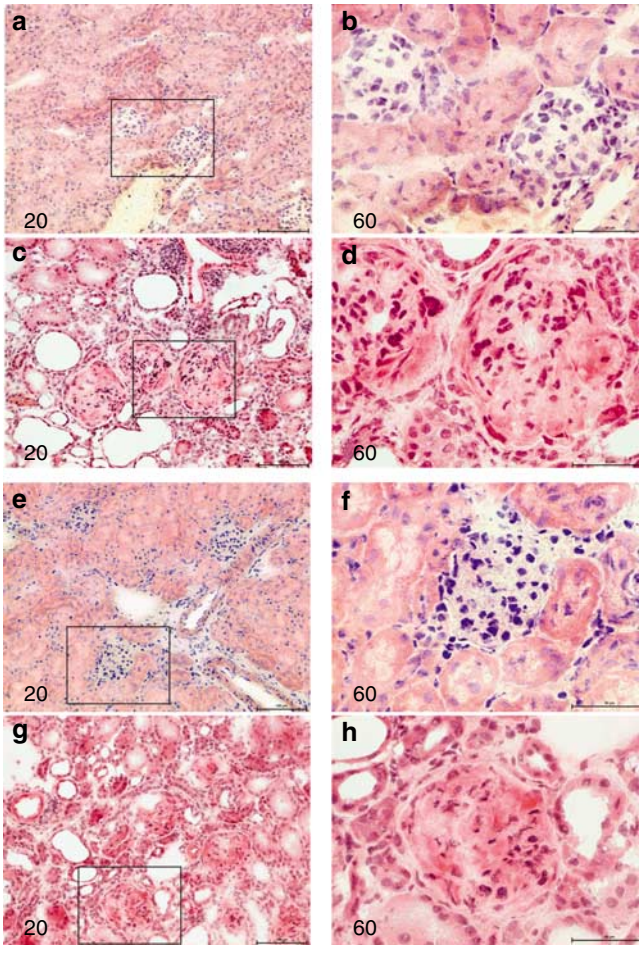


**Figure 1 | Histological comparison of kidneys from 'chronic' and IFNα-induced 'acute', nephritic NZB/W mice.** Views of the kidney (hematoxylin stained) of a spontaneously nephritic NZB/W mouse (**c**, **d**); comparison with the kidney of a 4-week-old NZB/W mouse (**a**, **b**). Views of the kidney of a nephritic, IFNα-treated NZB/W mouse (**g**, **h**); comparison with the kidney of a NZB/W mouse treated with the control Adv (**e**, **f**).

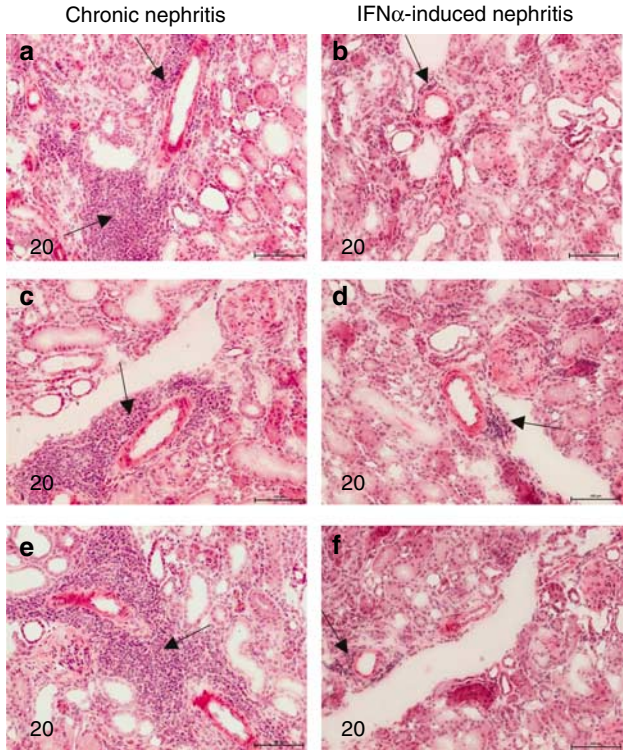


**Figure 2 | Histological comparison of interstitial infiltration in kidneys from 'chronic' and IFNα-induced 'acute', nephritic NZB/W mice.** (**a**, **c, e**) Views of perivascular interstitial infiltrates in the kidney (hematoxylin stained) of three individual spontaneously nephritic NZB/W mice ; (**b, d, f**) view of the renal interstitium of three individual, nephritic, IFNα-treated NZB/W mice. Note the large perivascular infiltrates in the kidney of spontaneously nephritic NZB/W mice (arrows) (**c**) compared with the virtual absence of these infiltrates in IFNα-treated NZB/W mice (**b, d, f**).

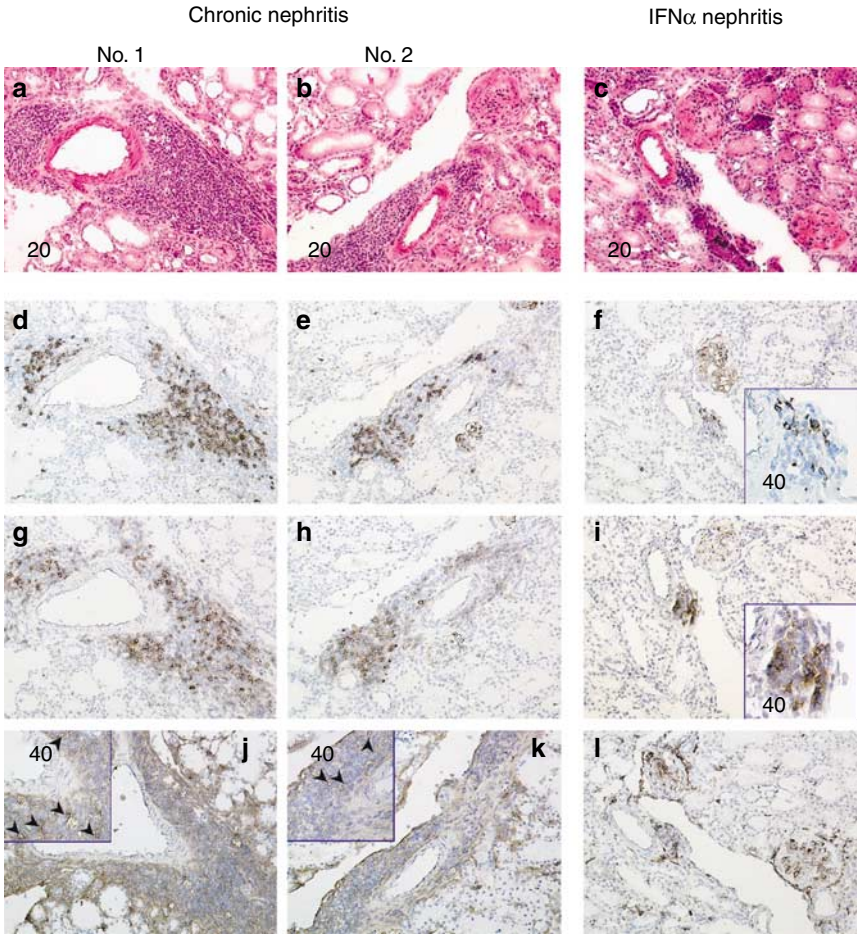


**Figure 3 | Immunohistochemical comparison of interstitial infiltration in kidneys from 'chronic' and IFNα-induced 'acute', nephritic NZB/W mice.** Hematoxylin stained views of perivascular infiltrates in the kidney of 2 individual spontaneously nephritic NZB/W mice (**a**, **b**) and one nephritic, IFNα-treated mouse (**c**). Immunohistochemical staining of kidney sections from two individual spontaneously nephritic NZB/W mice, and one IFNα-treated, nephritic mouse, stained for B lymphocytes (**d–f**), T lymphocytes (**g–i**), and myeloid cells (**j–l**).

**Table 1 | Migration of circulating leukocytes in lupus nephritis**

| | Balb/c | NZB/W |
|---|---|---|
| CD19+ | 8±3 | 34±11* |
| CD3+ | 11±4 | 50±12* |
| Mac-1+ | 0 | 24±3* |

NZB/W, New Zealand Black/White.
Fluorescently labeled spleen cells from 7- to 9-month-old Balb/c or NZB/W mice were injected to 7- to 9-month-old Balb/c or NZB/W mice, respectively. The numbers of fluorescently labeled CD19+, CD3+, and Mac1+ cells per $10^6$ lymphocytes in recipient kidneys were assessed by flow cytometry. Results are expressed as the mean±s.e.m., and are from eight independent experiments.
*$P<0.05$, Mann–Whitney *U*-test.

diseased NZB/W mice (Figure 4b, d, f). Both B and T lymphocytes and myeloid cells from diseased NZB/W exhibited a migration capacity ~2- to 3-fold higher in diseased recipient mice than in young mice. These fluorescent leukocytes were visualized mainly in interstitial areas (Figure 5). Of note, the prevalence of the original populations of B cells, T cells, and myeloid cells within transferred spleen cells was similar between young and spontaneously nephritic NZB/W mice (Table 2). Thus, both leukocyte and kidney abnormalities synergistically contribute to the increased recruitment of circulating leukocytes in NZB/W mice with lupus nephritis.

### Normal migration of NZB/W leukocytes to lymphoid organs

We tested the ability of leukocytes from NZB/W mice with lupus nephritis to migrate to organs other than the kidney (ie, to lymphoid organs). Migration of leukocytes from control Balb/c mice was studied in parallel. As shown in Table 3 (where findings for B lymphocytes are reported), high numbers of leukocytes migrated to lymphoid organs, both in controls and in NZB/W mice. Nevertheless, migration of B lymphocytes from either young or diseased NZB/W mice into the lymphoid organs of diseased recipients did not differ from that observed in Balb/c mice. Similar findings were observed for T lymphocytes and myeloid cells (data not shown). Thus, the increased ability of leukocytes from nephritic NZB/W mice to migrate to tissues is restricted to the kidney.

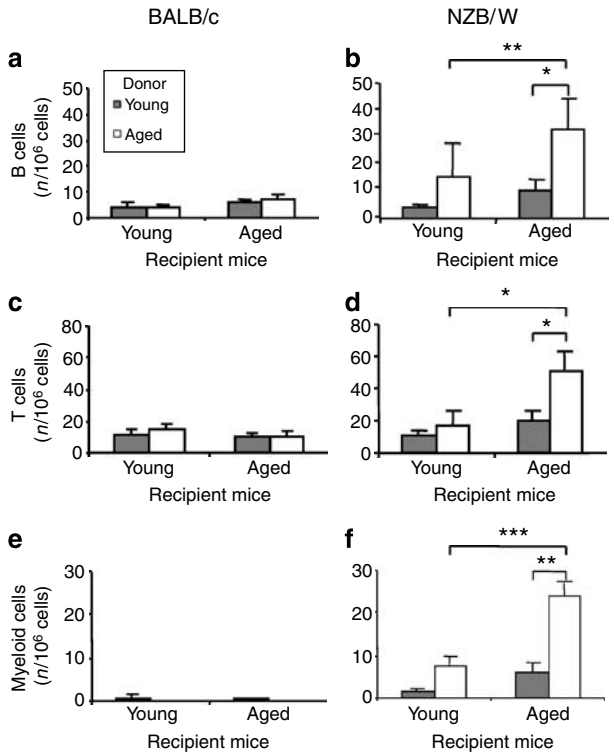


**Figure 4 | Role of leukocyte and kidney abnormalities in leukocyte recruitment in lupus nephritis.** Spleen cells from young (7–10 weeks) or aged (7–9 months) normal mice (**a**–**e**) or nephritic mice (**b**–**f**) were labeled and injected to either young or aged, syngeneic mice, respectively. Migration of B lymphocytes (CD19+ cells), (**a**, **b**) T lymphocytes (CD3+ cells) (**c**, **d**), and myeloid cells (CD11b+ cells) (**e**, **f**) was analyzed 18 h later by flow cytometry. Results are the mean ± s.e.m. of labeled cells per $10^6$ lymphoid cells in the recipient kidneys, and are from eight independent experiments.

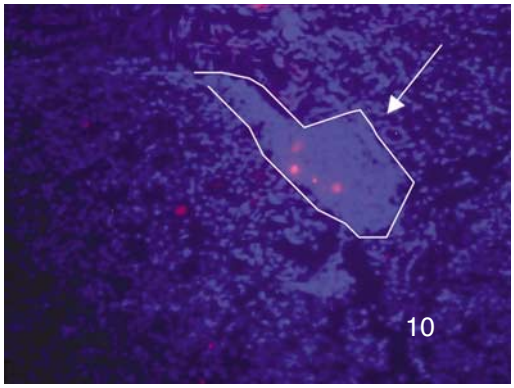


**Figure 5 | Migration of leukocytes in kidneys from spontaneously nephritic NZB/W mice.** Spontaneously nephritic NZB/W mice were injected intravenously with syngeneic leukocytes from nephritic mice, and were killed 18 h after injection. Kidney sections were stained with DAPI (blue) and observed for the presence of fluorescent migrated cells (pink). Note that the fluorescent cells are mainly located in the interstitium.

### Lack of interstitial infiltrates in kidneys from nephritic IFNα-treated NZB/W mice

We recently developed an experimental model of lupus in which systemic IFNα *in vivo* induces rapid and lethal lupus nephritis in young NZB/W, but not in Balb/c, mice.[18] We therefore assessed the nature of kidney lesions in proteinuric, IFNα-treated mice. As in the spontaneous model, histological staining revealed a severe diffuse proliferative glomerulonephritis with either segmental or global involvement of all glomeruli (Figure 1g and h). Wire loop lesions and segmental glomerulosclerosis could also be identified. However, at variance with the spontaneous model, there were very little infiltrating leukocytes in the interstitium (Figure 2b, d, f), which appeared to be predominantly T cells (vs B cells in the chronic model) (compare panels f and i in Figure 3 with panels d, e, g, h). Of note, nephritic IFNα-treated mice showed a significant number of myeloid cells scattered all over the renal interstitium (Figure 3l).

### Leukocytes from nephritic IFNα-treated NZB/W do not migrate to inflamed lupus kidneys

We asked whether IFNα treatment of NZB/W mice modified the homing properties of their spleen leukocytes, which might account for the absence of leukocyte infiltration in the renal interstitium. Fluorescently labeled leukocytes from nephritic IFNα-treated mice were injected in spontaneously nephritic NZB/W recipients. Leukocytes from NZB/W mice treated with a control adenovirus (Adv) were studied in parallel. The prevalence of the original populations of B cells, T cells, and myeloid cells within the transferred spleen cells was similar between control Adv-treated and IFNα Adv-treated, NZB/W mice (Table 2). Lymphocytes from IFNα-treated mice did not migrate to the kidney of nephritic NZB/W mice (Table 4). There was also no increased migration of myeloid cells as compared with controls. Thus, IFNα treatment of young NZB/W mice although triggering acute and lethal lupus nephritis, does not increase the potency of leukocytes to migrate to inflamed kidneys.

Conversely, we tested whether the kidney from nephritic IFNα-treated mice attracts leukocytes from spontaneously nephritic NZB/W mice. The kidney from IFNα-treated mice had a capacity similar to that of spontaneously diseased NZB/W mice to attract leukocytes from the latter (Figure 6a and b). The frequency of B and T lymphocytes as well as that of myeloid cells (Figure 6c) that migrated to the kidney was similar when comparing kidneys from the 'acute' and the 'chronic' model of lupus nephritis.

### Leukocytes from IFNα-treated and spontaneously nephritic NZB/W mice display similar levels of activation

To test whether the inability of leukocytes from IFNα-treated mice to migrate into the inflamed kidney reflected impaired activation of 'IFNα' leukocytes, we compared the levels of spleen leukocyte activation markers in the 'chronic' and 'acute' models of disease. T cells from IFNα-treated mice displayed similar (or even higher) levels of the T lymphocyte CD25 and CD69 activation markers than those from spontaneously nephritic NZB/W (Figure 7a). Likewise, CD86 levels on B cells as well as the frequency of CD19+CD138+ plasma cells were comparable between

**Table 2 | Composition of lymphoid and myeloid cells in spleens of donor mice**

| Donor | Numbers of cells/$10^6$ total number of spleen cells | | |
|---|---|---|---|
| | B cells | T cells | Myeloid cells |
| Young BALB/c (*n*=5) | 371 354 ± 24 127 | 418 487 ± 31 543 | 50 289 ± 7289 |
| Aged BALB/c (*n*=5) | 403 229 ± 38 525 | 389 290 ± 34 011 | 46 017 ± 7783 |
| Young NZB/W (*n*=5) | 414 076 ± 15 691 | 372 572 ± 37 044 | 39 050 ± 12 569 |
| Aged NZB/W (*n*=8) | 438 380 ± 25 362 | 327 211 ± 39 597 | 47 367 ± 4170 |
| Null Adv NZB/W (*n*=3) | 453 910 ± 16 356 | 399 804 ± 41 736 | 39 402 ± 3656 |
| IFNα Adv NZB/W (*n*=3) | 442 434 ± 27 437 | 347 191 ± 18 962 | 66 748 ± 20 718 |

Adv, adenovirus; IFNα, interferon-α; NZB/W, New Zealand Black/White.
The respective cell representation of B, T, lymphocyte and myeloid cells among splenocytes from donor mice was determined. Results are expressed as the mean cell number ± s.d. of a given population per $10^6$ total spleen cells.
$P > 0.34$ for all comparisons.

**Table 3 | Homing of B lymphocytes to lymphoid organs**

| Recipient mice | Labeled CD19+ cells (n/$10^6$ cells) | |
|---|---|---|
| | Young donor | Aged donor |
| *BALB/c* | | |
| Spleen | 4297 ± 486 | 5142 ± 1203 |
| Bone marrow | 408 ± 62 | 528 ± 128 |
| Lymph nodes | 829 ± 242 | 765 ± 429 |
| *NZB/W* | | |
| Spleen | 2508 ± 503 | 3583 ± 757 |
| Bone marrow | 439 ± 108 | 391 ± 71 |
| Lymph nodes | 471 ± 138 | 703 ± 259 |

NZB/W, New Zealand Black/White.
Spleen cells from young or aged mice were fluorescently labeled and injected to syngeneic, aged mice. Migration of fluorescently labeled B lymphocytes (CD19+ cells) to lymphoid organs was analyzed by flow cytometry. Results are expressed as the mean ± s.e.m. of labeled CD19+ cells per $10^6$ lymphocytes of the recipient organ, and are from eight independent experiments.
$P > 0.1$ for all comparisons.

**Table 4 | Migration to kidneys of leukocytes from IFNα-treated NZB/W mice**

| | Adv-Null NZB/W | Adv-IFNα NZB/W |
|---|---|---|
| CD19+ | 11 ± 2 | 5 ± 2 |
| CD3+ | 33 ± 8 | 26 ± 4 |
| Mac-1+ | 7 ± 4 | 7 ± 3 |

Adv, adenovirus; IFNα, interferon-α; NZB/W, New Zealand Black/White.
Fluorescently labeled spleen cells from NZB/W mice treated with either a control or an mIFNα-encoding Adv were injected to aged NZB/W mice. The numbers of fluorescently labeled CD19+, CD3+, and Mac1+ cells per $10^6$ lymphocytes in recipient kidneys were assessed by flow cytometry. Results are expressed as the mean ± s.e.m., and are from six mice in each group.
$P > 0.1$ for all comparisons.

the two models (Figure 7b). Thus, the lack of significant 'IFNα' leukocyte infiltration is not explained by impaired leukocyte activation.

### Differential expression of chemokines in the kidneys of IFNα-treated and unmanipulated NZB/W mice

Since chemokines are involved in the recruitment of circulating leukocytes into the kidney,[20,21] we asked whether differential expression of renal chemokines could account for the differential leukocyte recruitment seen between the two models of lupus nephritis. Reverse transcription-polymerase

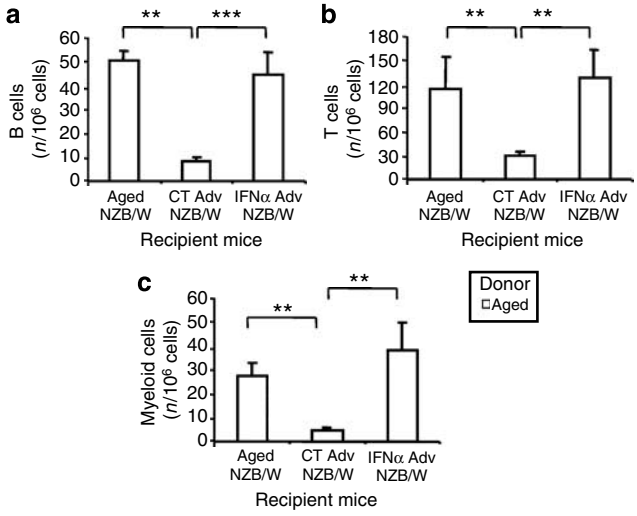


**Figure 6 | Leukocyte recruitment in kidneys from IFNα-induced lupus.** Spleen cells from spontaneously nephritic, aged, NZB/W mice were fluorescently labeled and injected to NZB/W recipients treated with control Adv or IFNα Adv, or to spontaneously nephritic mice. Migration of B lymphocytes (CD19 + cells) (**a**), T lymphocytes (CD3 + cells) (**b**), and myeloid cells (CD11b + cells) (**c**) was analyzed 18 h later by flow cytometry. Results are expressed as the mean ± s.e.m. of labeled cells per $10^6$ lymphoid cells in recipient kidneys, and are from six mice in each group.

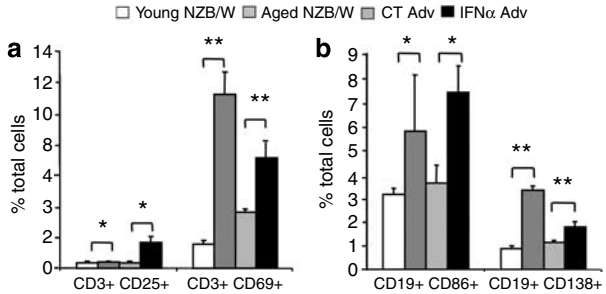


**Figure 7 | Leukocytes from both spontaneously and IFNα-treated, nephritic NZB/W mice are activated.** Spleen cells from nephritic, aged, NZB/W mice and from their respective controls (untreated or control Adv-treated, pre-autoimmune, mice) were phenotyped for surface activation markers using CD25, and CD69 for CD3 + T cells (**a**), and CD86 and CD138 for CD19 + B cells (**b**), respectively. Results are expressed as the mean % ± s.e.m. of activated cells vs total spleen lymphocytes, and are from six mice in each group.

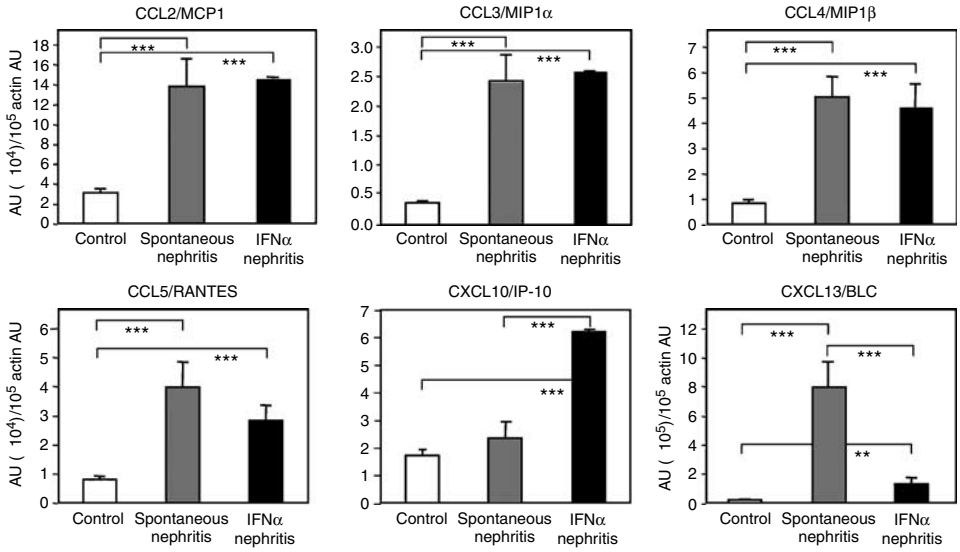


**Figure 8 | Reverse transcription-polymerase chain reaction analysis of renal chemokines in spontaneously and IFNα-treated, nephritic NZB/W mice.** Real-time reverse transcription-polymerase chain reaction was performed using total renal RNA of pre-autoimmune (white), spontaneously nephritic (gray), and IFNα-treated nephritic (black) NZB/W mice. Levels of mRNA expression for the indicated chemokines are expressed in relation to actin mRNA expression, and are expressed in arbitrary units (AU)/$10^5$ AU of actin. Results are the mean ± s.d. from 8–9 individual mice in each group. $^{**}P<0.01$; $^{***}P<0.005$.

chain reaction analysis of kidney samples showed that gene expression of the inflammatory chemokines CCL2/MCP-1, CCL3/MIP1α, CCL4/MIP1β, and CCL5/RANTES was similarly upregulated in kidneys from spontaneously and IFNα-treated, nephritic NZB/W mice compared with that in young mice (Figure 8). However, the expression of two chemokine genes differed between IFNα-treated and spontaneously diseased NZB/W mice. Expression of the IFN-inducible chemokine CXCL10/IP-10 was increased by >3-fold ($P<0.001$) in IFNα-treated mice compared with that in spontaneously nephritic mice. In the latter, CXCL10/IP-10 gene expression was slightly but not significantly increased compared with that in young pre-autoimmune controls. In contrast, CXCL13/BLC gene expression was markedly increased in spontaneously nephritic mice (by 7-fold; $P<10^{-4}$), compared to pre-autoimmune, control mice, whereas the increase was limited, though significantly ($P<0.005$), in IFNα-treated mice (Figure 8). Thus, the kidney from chronically and acutely nephritic mice shows both common and differential expressions of chemokine genes.

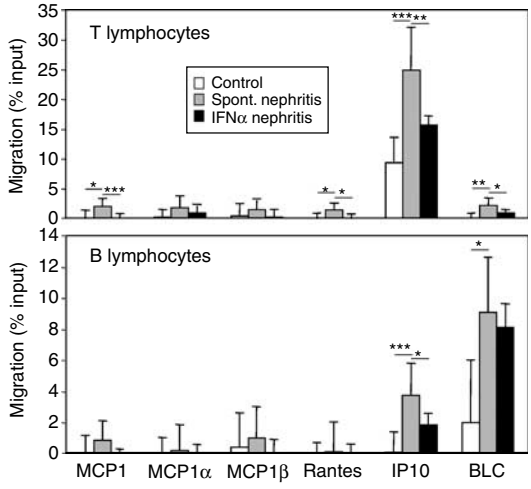


**Figure 9 | Migration of spleen cells to chemokines in spontaneously and IFNα-treated, nephritic NZB/W mice.** *In vitro* migration of T and B lymphocytes in response to various chemokines was tested. Results are expressed as the percentage of the total input cells that had migrated into the bottom chamber. For each value, we subtracted spontaneous migration of splenocytes in the mock control. Data are the mean ± s.d. from 4–8 individual mice in each group. $^{*}P<0.05$; $^{**}P<0.01$; and $^{***}P<0.005$.

### Migration of spleen cells to chemokines in spontaneously and IFNα-treated, nephritic NZB/W mice

To know whether the absence of inflammatory infiltrates in the kidney of IFNα-treated mice lies in the defects of leukocytes to migrate toward chemokines, we compared such migration, *in vitro*, on splenocytes from both chronic and acute models of lupus nephritis. Leukocytes from IFNα-induced, nephritic mice migrated less efficiently toward several inflammatory chemokines than leukocytes form spontaneously nephritic mice. Indeed, 'IFNα' T cells showed depressed chemotaxis to MCP-1 and RANTES ($P<0.01$ and $P<0.05$ vs spontaneous nephritis, respectively) and a trend to diminished chemotaxis to MIP1α and MIP1β (Figure 9). In regard to the expression of renal chemokines described above, migration of 'IFNα' T cells toward IP-10 was depressed when compared with the marked increased sensitivity of T cells from spontaneously nephritic mice ($P<0.01$), while chemotaxis of B cells to BLC was similarly

increased in both the models. Thus, both depressed renal chemokine expression (in particular, BLC) and leukocytic chemotaxis impairment (in particular to IP-10) may contribute to the paucity of interstitial inflammatory infiltration in acute IFNα-induced nephritis.

## DISCUSSION

The severity of interstitial inflammation in lupus nephritis correlates with the degree of renal insufficiency and predicts progressive deterioration of renal function.[2,6–9] Little is known however about both the origin of mononuclear infiltrates and the factors that favor such infiltration. Here, we show in the NZB/W mouse model of lupus that B, T lymphocytes and myeloid cells from splenic origin can migrate to the kidney, and that this migration operates in restricted conditions that involve abnormalities in both leukocytes and the kidney.

Our *in vivo* transfer experiments showed that spleen leukocytes from spontaneously nephritic NZB/W have a trend to migrate into non-inflamed kidney of young NZB/W, an ability that is further potentiated by the inflamed state of the recipient kidney. Therefore, combined leukocyte and kidney abnormalities are needed for interstitial infiltration. All major cell populations of the spleen from chronically diseased mice showed an exclusive ability to migrate to the kidney and not to other target organs. This contrasts with peritoneal lupus B1 B cells that showed defective homing to the peritoneal cavity and preferential recruitment in several target organs of diseased NZB/W mice, including the kidney.[22,23] Using two models of lupus nephritis, one with chronic and the other with acute (IFNα induced) disease, we found that the latter differed from the former by the virtual absence of interstitial inflammatory infiltration. We took advantage of these models to further delineate kidney and/or leukocyte abnormalities that promote or hamper interstitial leukocyte infiltration.

The absence of leukocyte infiltration in the 'acute', IFNα-induced form of nephritis could result from particular features of the interstitial kidney compartment and/or circulating leukocytes. At the kidney level, our data show that leukocytes from old, proteinuric NZB/W mice are able to migrate *in vivo* into the kidney of proteinuric IFNα-treated mice, suggesting that the kidneys of both 'chronic' and 'acute' models of lupus nephritis share some common features that permit leukocyte migration in both instances. Expression of inflammatory chemokines (ie, CCL2/MCP-1, CCL3/MIP1α, CCL4/MIP1β, and CCL5/RANTES) is increased not only in spontaneously nephritic NZB/W mice, a result in accordance with previous reports in MRL *lpr* and NZB/W models of murine lupus nephritis,[24–27] but also in the kidney of IFNα-treated mice with acute nephritis. In human and murine lupus nephritis, overexpression of CCL2, CCL3, CCL4, and CCL5 is associated to macrophage and T-cell infiltration.[20,21] Similar overexpression of these chemokines in the kidney of spontaneously and IFNα-induced nephritic mice might explain the ability of monocytes and T cells from spontaneously diseased mice to migrate *in vivo* into 'IFNα' kidneys. Transfer experiments also showed the ability of B cells from spontaneously diseased mice to migrate *in vivo* into 'IFNα' kidneys. We found however that gene expression of BLC/CXCL13, a major B-cell attractant, was lower in IFNα kidneys than in kidneys of spontaneously nephritic mice in which BLC/CXCL13 gene expression was markedly increased compared with pre-autoimmune mice, as previously reported.[25,28] This suggests that either the levels of CXCL13/BLC in the 'IFNα' kidney are sufficient to attract B lymphocytes from spontaneously nephritic mice and/or that B cells are attracted by other kidney-expressed chemokines such as CXCL10/IP-10, whose corresponding receptor CXCR3 is expressed on activated normal B cells[29] and on most B cells from lupus patients.[30] The kidney of IFNα-treated mice strongly expressed the CXCL10/IP-10 gene, supporting the above hypothesis. Thus, differences in a very restricted set of renal chemokines (ie, CXCL10/IP-10 and BLC/CXCL13) exist between the chronic and acute model of lupus nephritis.

At the leukocyte level, our transfer experiments showed that leukocytes from acutely diseased mice failed to migrate *in vivo* into chronically inflamed kidney. This result is corroborated by *in vitro* migration experiments in which splenic T cells from IFNα-treated mice show poor, if any, migration toward major T-cell attractant chemokines including CCL2/MCP-1, CCL3/MIP1α, CCL4/MIP1β, CCL5/RANTES, and, to lesser extent, CXCL10/IP-10. Whether this is due to lack/internalization of the corresponding chemokine receptors and/or defects in signal transduction is unknown at present. Chemotaxis of 'IFNα' splenic B cells to CXCL13/BLC was upregulated as in the spontaneous model, suggesting that defective 'IFNα' B-cell migration *in vivo* may be due to insufficient expression of the chemokine at the kidney level.

Therefore, various mechanisms explain why acutely, IFNα-treated, nephritic mice show very little, if any, leukocyte infiltration in their kidney. At the kidney level, the little expression of CXCL13/BLC may not be sufficient to attract B lymphocytes, although these cells express a normal chemotactic response to the chemokine. Indeed, aberrant expression of CXCL13 is sufficient to induce the formation of ectopic lymphoid tissue in non-lymphoid organs,[31] and may be responsible for the renal recruitment of B cells and activated T cells that express the CXCL13 receptor, CXCR5, leading to renal inflammation in spontaneously nephritic NZB/W mice[28] and in patients with acute interstitial nephritis.[32] On the other hand, the poor or no chemotactic response of 'IFNα' T cells to inflammatory T-cell attractant chemokines might explain the lack of T-cell infiltration in these mice. Thus, combined abnormalities in both kidney-expressed chemokines and leukocyte chemotactic activity may concur to the development or, alternatively, the absence of interstitial inflammation.

The use of our 'acute' model of lupus will be highly informative to dissect the inflammation mechanisms

involved in glomeruli against interstitial damages. Acute pathological changes induced by IFNα, with prominent glomerular damages, might be close to those observed during 'flares' of lupus nephritis in human, with reversion following aggressive immunosuppressive treatments, whereas changes in the 'chronic' form would be more representative of lesions leading to progressive fibrosis in human lupus nephritis. Thus, delineation of the immune pathologic chemokine/chemokine receptors network in both the 'acute' and 'chronic' models of lupus will be important to determine whether similar chemokine/chemokine receptor antagonists should be tested depending on the stage of nephritis and on the final purpose of the treatment, to cure a flare or to prevent fibrosis.

## MATERIALS AND METHODS

### Mice

Female (NZB × NZW) F1 (NZB/W) mice were bred in our conventional animal facility. Age-matched female BALB/c mice were used as controls. NZB/W mice (7- to 9-months old) with moderate to severe proteinuria (see below) were considered aged, while 7- to 10-week-old mice without proteinuria were considered young.

### Proteinuria

Urine was tested for proteinuria using Multistix 8 SG (Bayer Diagnostics, Puteaux, France) on a 0–4+ scale, corresponding to the following approximate protein concentrations: 0, negative or trace; 1+, 30 mg/dl; 2+, 100 mg/dl; 3+, 300 mg/dl; and 4+, 2000 mg/dl. Mice were considered to have nephritis if two consecutive urine samples scored 3+.

### IFNα treatment

The recombinant adenovector (Adv) containing the mIFNa (AdCMVmIFNα5) and the control (AdvCMV null) were obtained from Qbiogene (Illkirch, France). NZB/W mice were treated at 10 weeks of age with a single intravenous injection of $1 \times 10^{10}$ IFNα Adv particles in the retro-orbital plexus.[18] Control NZB/W mice received the same amount of control Adv particles. A total of 3–4 weeks following initiation of IFNα Adv treatment, mice became proteinuric with scores of 3+–4+.[18]

### Cell labeling and adoptive transfer

Splenocytes were labeled using the cell tracker orange CMTMR or Calcein AM (Molecular Probes, Strasbourg, France). For each adoptive transfer, spleen cells were labeled in 10 μM of cell tracker for 30 min at 37°C. A 200-μl volume of $2.5 \times 10^7$/ml cells was labeled with Calcein, while another group of cells were labeled with CMTMR. Equal numbers of each labeled cells were co-injected intravenous into syngeneic mice, which were killed 18 h after injection for quantifying cell migration by flow cytometry.

### Flow cytometry studies

Single-cell preparations from recipient mice were stained with specific antibodies (Abs) (all from BD Pharmingen, Le Pont de Chaix, France) and analyzed by four-color flow cytometry. Antibodies were PerCP-Cy5,5-labeled rat anti-CD11b, PerCP-Cy5,5-labeled hamster anti-CD3, allophycocyanin (APC)-conjugated rat anti-CD19, and the corresponding isotype-matched control Abs. The frequency of cells that had migrated into the recipient tissues is expressed as the numbers of fluorescently labeled cells for $10^6$ lymphocytes counted in the lymphocyte gate. Activation of B, T lymphocytes, and macrophages was examined using the following Abs: fluorescein isothiocyanate-labeled, rat anti-CD25, rat anti-CD86, biotin-labeled, hamster anti-CD69, and rat anti-CD138, and the corresponding isotype-matched control Abs.

### Immunohistochemistry

Cryostat sections (5-μm thick) of kidney were fixed in ice-cold acetone, air dried, hydrated with phosphate-buffered saline, blocked with the avidin–biotin kit (Vector Laboratories, Burlingame, CA, USA), followed by treatment with normal rabbit serum (or goat serum for anti-CD3 staining), and were then stained with anti-B220, anti-CD11b or anti-CD3 monoclonal antibodies (all from BD Pharmingen) for 1 h at room temperature. Detection of bound Abs was performed using the rat IgG Vectastain Elite ABC kit (Vector Laboratories), except for CD3 staining which was revealed using biotinylated goat anti-hamster Abs (Jackson) followed by peroxidase-conjugated streptavidin (Roche Diagnostics, Meylan, France). Color was developed with 3,3′-diaminobenzidine (Vector Laboratories) and samples counterstained with hematoxylin.

### Reverse transcription polymerase chain reaction analysis of chemokine expression

Total RNA was isolated from cryosections using RNeasy mini kit (Qiagen, Courtaboeuf, France). mRNAs were extracted using the oligotex kit (Qiagen), reverse transcribed using SuperScript II RT (Invitrogen, Cergy Pontoise, France), and amplified with specific oligonucleotide primers (CCL2: sense 5′-AGGTCCCTGTC ATGCTCTG-3′, antisense, 5′-TCTGGACCCATTCCTTCTTG-3′; C CL3: sense 5′-GCCCTTGCTGTTCTTCTCTGT-3′, antisense, 5′-GG CATTCAGTTCCAGGTCAGT-3′; CCL4: sense 5′-CCCACTTCC TGCTGTT-TCTC-3′, antisense, 5′-GAGGAGGCCTCTCCTGAA GT-3′; CCL5: sense 5′-CACCTGCCTC-ACCATATGGC-3′, antisense, 5′-GGCGGTTCCTTCGAGTGACA-3′; CXCL10: sense 5′-GC-TG CCGTCATTTTCTGC-3′, antisense, 5′-TCTCACTGGCCCGTCAT C-3′; CXCL13: sense 5′-CATAGATCGGATTCAAGTTACGCC-3′, antisense, 5′-TCTTGGTCCAGATCACAACTTCA-3′).

### *In vitro* migration assays

Migration of leukocytes was evaluated in duplicates using 96-well Transwell inserts (5 μm pore size; Corning Costar, Avon, France). In brief, spleen cells were first labeled with fluorescein isothiocyanate-labeled anti-CD3, phycoerythrin-labeled anti-B220, and APC-labeled CD11b monoclonal antibodies (BD Pharmingen). After washing, cells were resuspended into prewarmed migration medium (RPMI 1640, 20 mM Hepes, 1% bovine serum albumin), and 150 μl of the cell suspension (input of $2 \times 10^6$ cells) were added to the upper chamber of the insert. The lower chamber contained prewarmed migration medium with or without 500 ng/ml of the indicated recombinant mouse chemokine (R&D Systems, Lille, France). Cells that had migrated 3 h later into the lower chamber were counted by flow cytometry.

### Statistical analysis

Statistical analysis was performed using the non-parametric Mann–Whitney *U*-test.

## ACKNOWLEDGMENTS

This work was supported by the European Union FP6 (INNOCHEM, grant number LSHB-CT-2005-518167).

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