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Akiyama Y[1], Morikawa T[2], Maeda D[3], Niimi A[1], Nomiya A[4], Igawa Y[5], Fukayama M[2], Homma Y[1]
1. Department of Urology, Graduate School of Medicine, The University of Tokyo, 2. Department of Pathology, Graduate School of Medicine, The University of Tokyo, 3. Department of Cellular and Organ Pathology, Graduate School of Medicine, Akita University, 4. Department of Urology, Mitsui Memorial Hospital, 5. Department of Continence Medicine, Graduate School of Medicine, The University of Tokyo

INCREASED CXCR3 EXPRESSION OF INFILTRATING PLASMA CELLS IN HUNNER TYPE INTERSTITIAL CYSTITIS

Hypothesis / aims of study
An up-regulated CXCR3 pathway and affluent plasma cell infiltration are characteristic features of Hunner type interstitial cystitis (HIC) (1, 2). We examined the relationship of these two features using non-specific cystitis specimens as a control.

Study design, materials and methods
Bladder biopsy samples were taken from 27 patients with HIC (one each from a Hunner lesion and a non-lesion area (NLA), totalling 54 samples) and 15 patients with non-specific chronic cystitis (11 samples from 7 patients with non-specific chronic cystitis, and 12 samples from non-cancerous areas of 8 patients with non-muscle invasive bladder cancer). Diagnosis of HIC and non-specific chronic cystitis was made according to clinical guidelines for interstitial cystitis and hypersensitive bladder syndrome (3) and histological evidence of non-malignant chronic inflammation represented by predominant stromal infiltration of lymphoplasmacytic cells, edema, and fibrosis. We performed immunohistochemical detection of infiltrating T-lymphocytes, B-lymphocytes, plasma cells and CXCR3-positive cells using CD3, CD20, CD138 and CXCR3 antibodies, respectively. The number of CD3-positive T-lymphocytes, CD20-positive B-lymphocytes, CD138-positive plasma cells, and CXCR3-positive cells was quantified by digital image analysis (2). Double-immunofluorescence for CXCR3 and CD138 was used to detect CXCR3 expression pattern in plasma cells. Correlations between CXCR3 positivity and clinical parameters were explored.

Table 1. Demographics and characteristics in patients with HIC

	mean $\pm$ SD [range]
No. (male / female)	27 (3 / 24)
Mean age at the time of biopsy (years)	68.4 $\pm$ 11.4 [38 – 88]
Age at onset of IC (years)	65.1 $\pm$ 10.5 [38 - 80]
Years from symptom onset to biopsy (years)	3.3 $\pm$ 2.6 [0 - 8]
OSSI†	13.1 $\pm$ 4.1 [7 - 20]
OSPI†	11.4 $\pm$ 3.8 [3 - 16]
VAS†	6.4 $\pm$ 2.4 [1 - 10]
Urinary frequency (/day)	16.3 $\pm$ 5.7 [7 – 30]
Maximum voided volume (mL)	163.8 $\pm$ 59.6 [50 - 300]
Maximum bladder capacity at hydrodistension (mL)	521.2 $\pm$ 181.8 [200 – 900]

†OSSI / OSPI: O'Leary and Sant's symptom index and problem index, VAS: visual analogue scale (for pain)

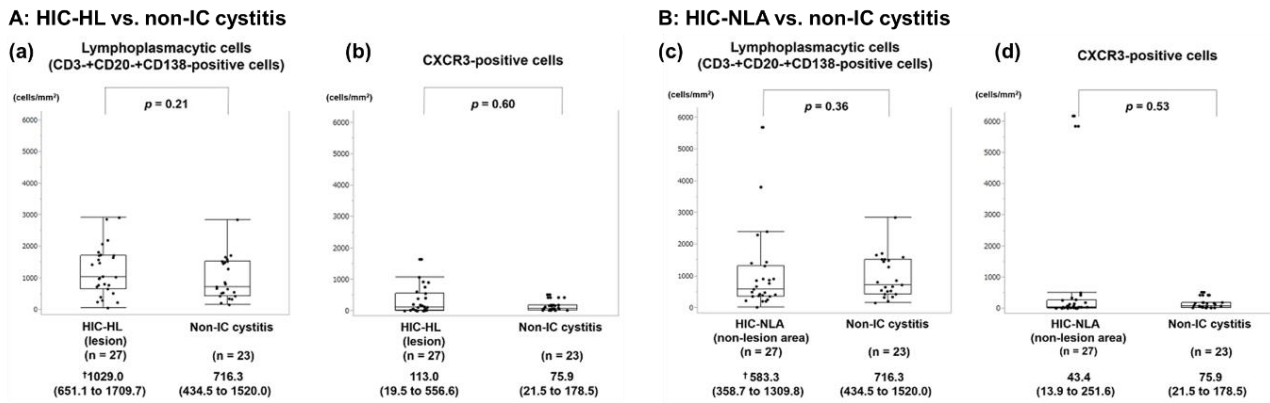Table 2. Correlation among cell numbers in bladder biopsy samples

(cells/mm²)	CXCR3-positive cells			CD3-positive T cells			CD20-positive B cells			CD138-positive plasma cells			Lymphoplasmacytic cells‡		
	HIC (HL)†	HIC (NLA)	Non-IC cystitis	HIC (HL)	HIC (NLA)	Non-IC cystitis	HIC (HL)	HIC (NLA)	Non-IC cystitis	HIC (HL)	HIC (NLA)	Non-IC cystitis	HIC (HL)	HIC (NLA)	Non-IC cystitis
CXCR3-positive cells				**0.34** (0.04)	**0.40** (0.04)	**0.51** (<0.01)	**0.58** (<0.01)	**0.48** (0.02)	0.33 (0.07)	**0.48** (0.01)	**0.47** (0.01)	-0.11 (0.62)	**0.56** (0.01)	**0.45** (0.01)	**0.46** (0.02)
CD3-positive cells							**0.62** (<0.01)	**0.66** (<0.01)	**0.54** (0.01)	**0.44** (0.02)	**0.68** (<0.01)	0.22 (0.58)	**0.82** (<0.01)	**0.80** (<0.01)	**0.91** (<0.01)
CD20-positive B cells										**0.43** (0.02)	**0.41** (0.04)	**0.40** (0.02)	**0.91** (<0.01)	**0.82** (<0.01)	**0.76** (<0.01)
CD138-positive plasma cells													**0.68** (<0.01)	**0.72** (<0.01)	**0.34** (0.04)

Values in the upper and lower rows indicate Spearman's correlation coefficient ρ and *P*-value (in the parentheses), respectively. Red when *P* < 0.05
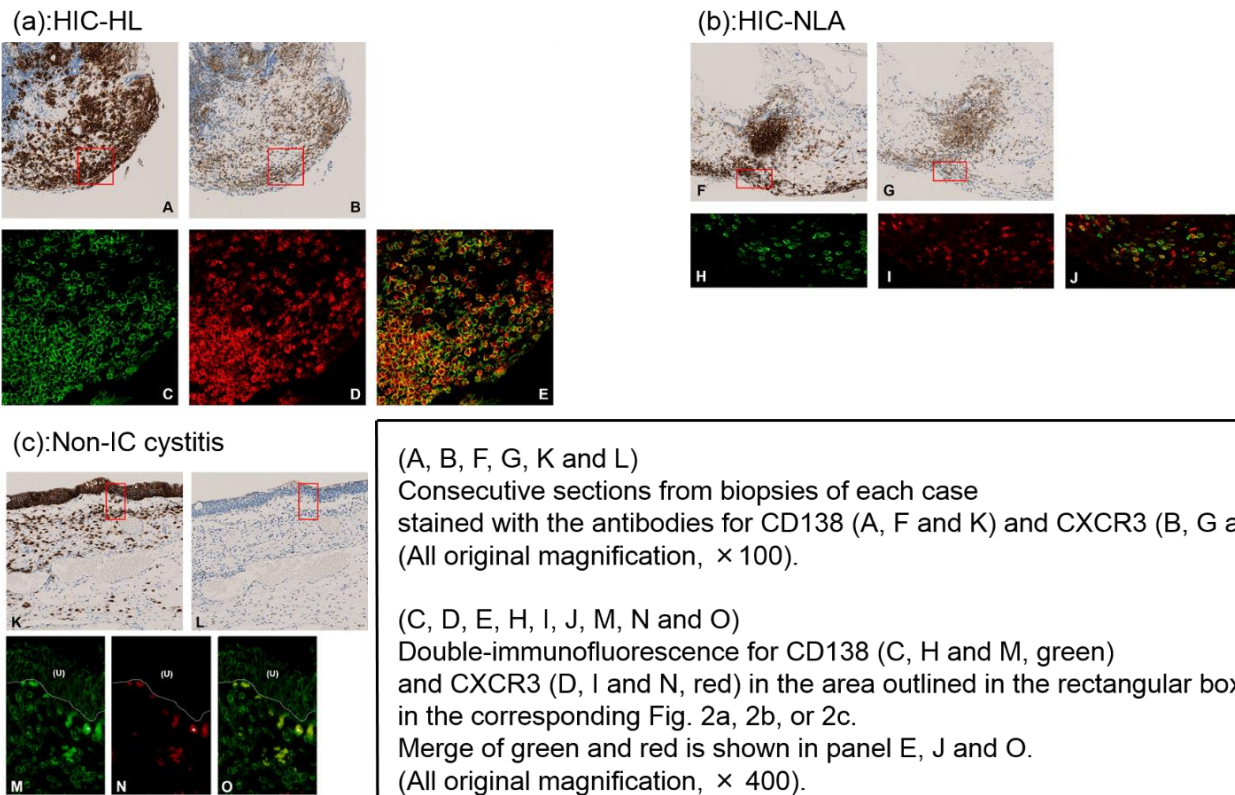†Number of samples: 27 for HIC (HL), 27 for HIC (NLA) and 23 for Non-IC cystitis
‡Lymphoplasmacytic cells: Sum of the CD3-positive cells, CD20-positive cells and CD138-positive cells

Fig. 1. Quantification of lymphoplasmacytic cells and CXCR3-positive cells by image analysis software

A: HIC-HL vs. non-IC cystitis

(a) Lymphoplasmacytic cells (CD3-+CD20-+CD138-positive cells) — $p = 0.21$

	HIC-HL (lesion) (n = 27)	Non-IC cystitis (n = 23)
	†1029.0 (651.1 to 1709.7)	716.3 (434.5 to 1520.0)

(b) CXCR3-positive cells — $p = 0.60$

	HIC-HL (lesion) (n = 27)	Non-IC cystitis (n = 23)
	113.0 (19.5 to 556.6)	75.9 (21.5 to 178.5)

B: HIC-NLA vs. non-IC cystitis

(c) Lymphoplasmacytic cells (CD3-+CD20-+CD138-positive cells) — $p = 0.36$

	HIC-NLA (non-lesion area) (n = 27)	Non-IC cystitis (n = 23)
	†583.3 (358.7 to 1309.8)	716.3 (434.5 to 1520.0)

(d) CXCR3-positive cells — $p = 0.53$

	HIC-NLA (non-lesion area) (n = 27)	Non-IC cystitis (n = 23)
	43.4 (13.9 to 251.6)	75.9 (21.5 to 178.5)

(a): The number of lymphocytes and plasma cells in HIC-HL and non-IC cystitis specimens.
(b): The number of CXCR3-positive cells in HIC-HL and non-IC cystitis specimens.
(c): The number of lymphocytes and plasma cells in HIC-NLA and non-IC cystitis specimens.
(d): The number of CXCR3-positive cells in HIC-NLA and non-IC cystitis specimens.
Values are expressed as median (Interquartile range).

Fig. 2 Representative images of localization of CD138-positive cells and CXCR3-positive cells in HIC-HL (a), HIC-NLA (b) and non-IC cystitis (c).

(a):HIC-HL

(b):HIC-NLA

(c):Non-IC cystitis

(A, B, F, G, K and L)
Consecutive sections from biopsies of each case
stained with the antibodies for CD138 (A, F and K) and CXCR3 (B, G and L).
(All original magnification, × 100).

(C, D, E, H, I, J, M, N and O)
Double-immunofluorescence for CD138 (C, H and M, green)
and CXCR3 (D, I and N, red) in the area outlined in the rectangular box
in the corresponding Fig. 2a, 2b, or 2c.
Merge of green and red is shown in panel E, J and O.
(All original magnification, × 400).

Results
Demographics and characteristics in patients with HIC are shown in Table 1. Gender distribution showed significant female predominance in the HIC group (24 versus 3) compared with the non-IC group (7 versus 8) ($p < 0.01$). The mean age was 68.4 (range 38 – 88) and 72.5 (range 54 – 85) years in the HIC and non-IC cystitis groups, respectively ($p = 0.26$). The density of CXCR3-positive cells showed no significant differences between HIC and non-specific cystitis specimens (Fig. 1). However, distribution of CXCR3-positivity in plasma cells indicated co-localization of CXCR3 with CD138 in HIC specimens, but not in non-specific cystitis specimens (Fig. 2). The number of CXCR3-positive cells correlated with plasma cells in HIC specimens alone (Table 2). No significant correlations between CXCR3-postive cell numbers and clinical parameters were observed (data not shown).

Interpretation of results
The results suggest that infiltration of CXCR3-positive plasma cells is a characteristic feature of HIC.

Concluding message
The CXCR3 pathway and specific immune responses may be involved in accumulation/retention of plasma cells and pathophysiology of the HIC bladder.

References
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