Immunoglobulin and T-cell receptor gene rearrangement in Castleman's disease: molecular genetic analysis

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Aims: Castleman's disease (CD) is a rare heterogeneous disorder that is associated with an increased risk of developing lymphoma. Whether CD is primarily hyperplastic or neoplastic in origin is not yet clear. The aim of this study was to investigate CD further by determining the clonality status of its lymphocyte populations.
Methods and results: We reviewed 20 patients with CD, 15 with the hyaline-vascular type and five with the plasma cell type. Immunoglobulin (JH) and T-cell receptor (TCR) gene rearrangements were examined using polymerase chain reaction and Southern blotting techniques. B-lymphocyte clonality was also assessed by flow cytometry (FC) and by immunohistochemistry (IHC). The age range of the patients was 15–66 years: nine female and 11 male. Monoclonal rearrangement of the immunoglobulin (JH) gene was detected in only one case. No cases were positive for monoclonal rearrangement of the TCR gene. All of the cases except one were negative for immunoglobulin light chain restriction by both FC and IHC.
Conclusions: The lymphoid cells in CD are most commonly polyclonal in origin, which supports a non-neoplastic origin. However, rare cases may show lymphocyte monoclonality, which could represent the development of a neoplastic population. The latter cases should be followed closely.

Keywords: Castleman's disease, clonality, giant lymph node hyperplasia, molecular genetic analysis

Abbreviations: CD, Castleman's disease; FC, flow cytometry; IHC, immunohistochemistry; LCD, localized Castleman's disease; MCD, multicentric Castleman's disease; PCR, polymerase chain reaction; TCR, T-cell receptor

Introduction

Castleman's disease (CD), also known as angiofollicular lymphoid hyperplasia and giant lymph node hyperplasia, is a heterogeneous group of lymphoid proliferations of undetermined aetiology. Three histological variants have been described: hyaline-vascular, plasma cell and mixed. In addition, two clinical types have been described: localized (LCD) and multicentric (MCD).[1] LCD is generally curable by surgical excision,

regardless of the histological variant, whereas MCD often requires systemic therapy and has a poorer clinical outcome.[1] MCD may be further divided by clinical criteria into two subgroups: MCD with neuropathy [Peripheral neuropathy, Organomegaly, Endocrinopathy, Monoclonal gammopathy and Skin changes (POEMS)-associated or neuropathic] and MCD without neuropathy (non-neuropathic).[2] The histology of MCD is usually of 'mixed' type. It presents with widespread or generalized lymphadenopathy and multisystem involvement. It probably arises in the context of an immunoregulatory defect and it may result in the outgrowth of monoclonal lymphocyte populations, usually of B-cell type.[3,4] However, whether CD is primarily a hyperplastic or a neoplastic

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disorder is still a matter of controversy. Only a few studies have reported the results of molecular genetics analyses for lymphocyte monoclonality in CD and the findings are contradictory. In the present investigation, we examined lymphocyte monoclonality in CD of both LCD and MCD types, using a combination of immuno-histochemistry (IHC), flow cytometry (FC) and molecular genetics techniques.

Materials and methods

Medical records were examined for cases of CD at the Toronto General Hospital (now University Health Network), Toronto, Canada, and King Abdulaziz University Hospital (KAUH), Jeddah, Saudi Arabia. Twenty cases were selected (18 from Toronto General Hospital, two from KAUH) from over a 15-year period (1988–2001). HIV+ patients were excluded. The pathology slides for each case were reviewed by two pathologists (J.A-M., D.B.), and a histological subtype was assigned by consensus. IHC was carried out on formalin-fixed paraffin-embedded sections using the avidin–biotin peroxidase complex (ABC) technique. Antibody reagents directed against CD45, CD45RO, CD20, CD43 and κ and λ light chains were obtained from Dako-Cytomation (Carpinteria, CA, USA). FC was carried out on nine cases using reagents obtained from Beckman Coulter (Miami, FL, USA). Molecular genetic analyses were carried out by polymerase chain reaction (PCR) using snap-frozen tissue in 15 cases and formalin-fixed paraffin-embedded tissue in five cases.[5] For PCR analysis, 500 ng to 1 µg samples of DNA were amplified using the appropriate primers (see below), electrophoresed through a 2% agarose gel and visualized using ethidium bromide. Appropriate positive, negative and internal controls were run with each specimen. PCR analysis was carried out using primers specific for the Framework 3 (FR3A) and J consensus regions of the immunoglobulin heavy chain gene (JH), essentially as described by Trainor et al.[6] A second PCR analysis using consensus primers specific for Framework 256 regions and J consensus regions[7] was also used to increase the detection rate. Internal control primers were multiplexed with each of the above to ensure the presence of amplifiable DNA as previously described.[8] PCR for T-cell receptor (TCR) γ gene rearrangement was performed according to standard methods. Southern blot analyses (SB) were performed on high-molecular-weight DNA according to standard methods. Seven cases were examined by SB. DNA was digested using BglII, BamHI/HindIII and XbaI for the immunoglobulin heavy chain gene and with BamHI, HindIII and EcoRI restriction enzymes for the TCR β chain

gene, transferred to nylon membranes and hybridized with the J6 probe or the CB1 probe to examine the rearrangement status of the IgH gene and the TCRβ gene, respectively.

Results

CLINICAL FINDINGS

The age range of the patients was from 16 to 90 years. Eleven were male and nine were female. Seventeen patients had localized CD (age range 16–65 years). The sites of involvement for localized CD were abdomen ($n = 6$), mediastinum ($n = 5$), cervical lymph node ($n = 3$), axillary lymph node ($n = 2$) and inguinal lymph node ($n = 1$). Two of the patients had a concurrent non-haematopoietic neoplasm: one phaeochromocytoma and one colonic adenocarcinoma. Three patients had multicentric CD (age range 28–90 years). All three presented with fever, peripheral lymphadenopathy and hepatosplenomegaly. Two also had ascites.

MORPHOLOGIC FINDINGS

Histological sections from the LCD cases showed typical features of the hyaline-vascular variant in 15 cases (Figure 1A,B). These consisted of effacement of architecture, multiple, small to medium-sized follicles with regressively transformed germinal centres and concentrically organized mantle zones of small lymphocytes and hypervascular interfollicular areas. Two patients had the plasma cell variant, characterized by a marked, sometimes sheet-like proliferation of plasma cells in the interfollicular areas (Figure 1B,C). The three cases of MCD were all of the plasma cell variant.

IMMUNOPHENOTYPING

In all of the cases but one the plasma cells in the interfollicular areas showed a mixed pattern of staining for cytoplasmic κ and λ light chains. The one exception was the single case classified as the mixed cell variant of LCD, in which the plasma cells showed restricted staining for λ light chain. FC results were available for nine patients and no cases showed light chain restriction of surface immunoglobulin.

MOLECULAR STUDIES

The results of the gene rearrangement studies are summarized in Table 1. Monoclonal IgH gene rearrangement was detected in only one case (Figure 2A).
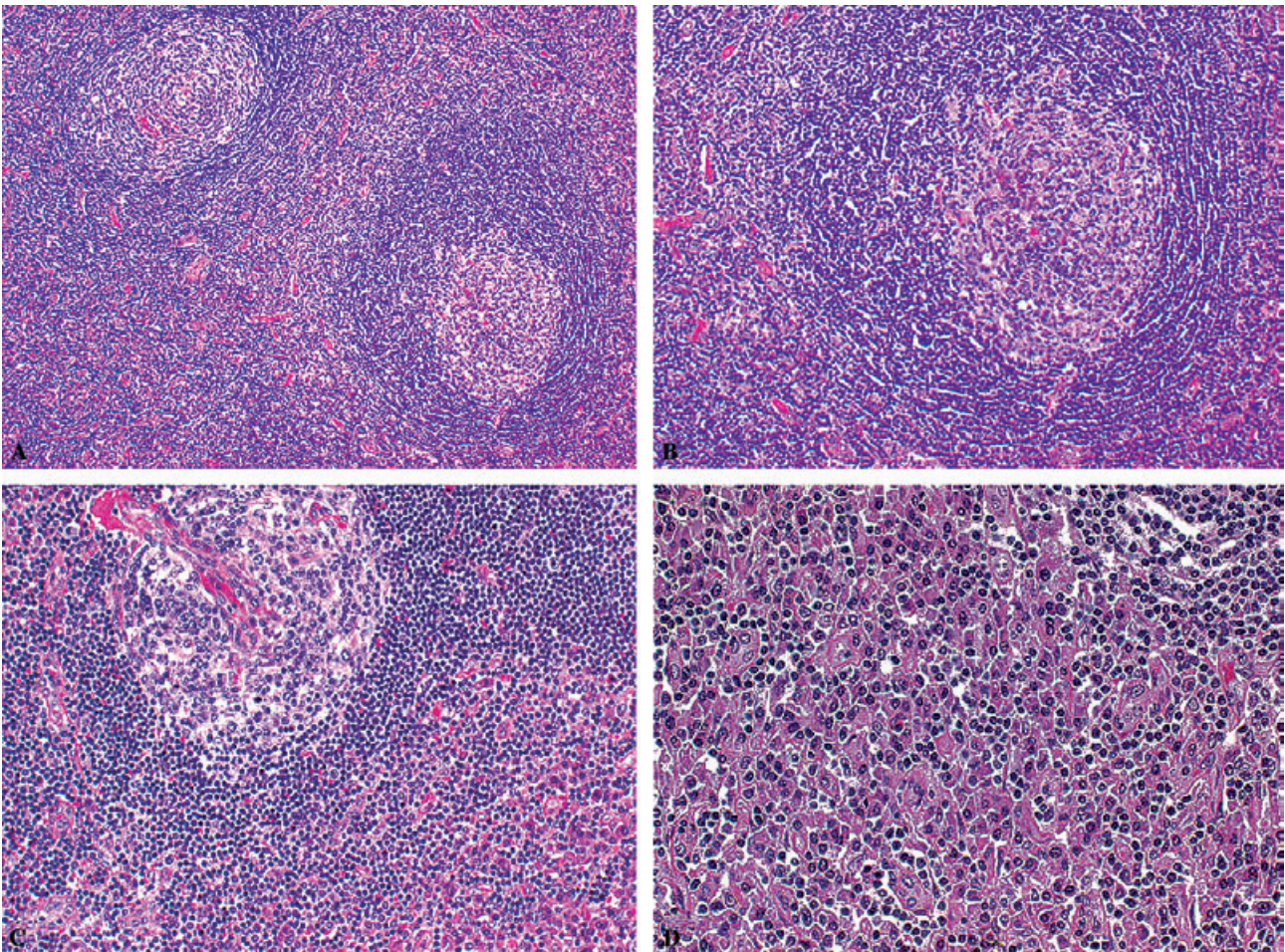
Figure 1. A, Section from medistinal lymph node reveals hypervascular follicles surrounded by a tight concentric layering of mantle-zone lymphocytes. **B,** Higher power from the same cases. **C,** Section from case 1 reveals a marked plasma cell infiltrate in the interfollicular regions and encasing the germinal centres. **D,** Higher power from case 1.

This was one of the two cases of LCD of the plasma cell variant. All of the others failed to show a monoclonal pattern, including the three cases of multicentric CD. Monoclonal rearrangement of the TCR γ gene was not detected in any case (Figure 2B). Thus a polyclonal pattern was seen in 19/20 cases for JH and in all 20 cases for TCRγ. None of the cases studied by SB showed rearrangement of the immunoglobulin heavy chain gene or the TCR gene (Figure 3).

Discussion

The detection of monoclonality in lymphoid proliferations is considered to be a strong indication of neoplasia. Molecular genetic analyses are the most recent and the most sensitive methods for determining lymphocyte monoclonality. Only a few previous studies have used molecular genetics methods to determine

lymphocyte clonality in CD and these have provided varying results.[4,9–12] Hanson *et al.*[10] detected monoclonal lymphocyte populations in three of four patients with MCD but in none of four patients with LCD. On the basis of these findings they suggested that MCD is distinct from LCD in that it may involve a clonal lymphoproliferation. Soulier *et al.*[12] reported molecular genetic analyses of 34 patients with CD. They detected monoclonal rearrangements of the IgH gene in only four of 30 patients with MCD and in none of four patients with LCD. Two of the cases with monoclonality had concurrent B-cell lymphoma and one had concurrent Hodgkin lymphoma, which suggested that the monoclonal populations may have been derived from these disorders rather than the CD. Only one case was positive for monoclonal rearrangement of the TCR gene, and this occurred in a patient with MCD who was HIV+. The authors concluded that in the large majority

Table 1. Summary of clinical and laboratory results of the patients with Castleman's disease

Case	Age/sex	Location	Diagnosis	Type	PCR/JH	PCR/TCR	SB	Flow cytometry	IHC
1	65/M	Paratracheal	LCD	PCS	+	–	ND	ND	I
2	90/M	Axilla	MCD	PCS	–	–	ND	CD3/19 = 80/20; CD4/8 = 70/10; K/L = 12/8	–
3	26/F	Paratracheal	LCD	HVV	–	–	ND	ND	–
4	26/F	Mediastinum	LCD	HVV	–	–	ND	ND	–
5	26/F	Mediastinum	LCD	HVV	–	–	ND	ND	–
6	38/F	Axilla	LCD	HVV	–	–	ND	ND	–
7	33/M	Inguinal	LCD	HVV	–	–	ND	CD3/19 = 60/40; CD4/8 = 53/7; K/L = 25/15	–
8	28/M	Axilla	MCD	PCS	–	–	ND	CD3/19 = 60/40; CD4/8 = 47/13; K/L = 25/15	–
9	32/F	Mediastinum	LCD	HVV	–	–	ND	ND	–
10	62/F	Neck	LCD	PCS	–	–	ND	ND	LLCR
11	54/M	Axilla	LCD	HVV	–	–	JH–, TCRB–	CD3/19 = 80/20; CD4/8 = 72/8; K/L = 11/9	–
12	41/M	Mesenteric	LCD	HVV	–	–	JH–, TCRB–	ND	–
13	51/M	Pericolic	LCD	HVV	–	–	ND	ND	–
14	15/M	Submandibular	LCD	HVV	–	–	JH–, TCRB–	CD3/19 = 65/35; CD4/8 = 42/20; K/L = 19/15	–
15	66/F	Omentum	LCD	HVV	–	–	JH–, TCRB–	CD3/19 = 30/70; CD4/8 = 24/5; K/L = 41/30	–
16	52/F	Axilla	MCD	PCS	–	–	JH–, TCRB–	CD3/19 = 65/35; CD4/8 = 57/9; K/L = 19/15	I
17	30/F	Abdomen	LCD	HVV	–	–	JH–, TCRB–	CD3/19 = 33/64; CD4/8 = 22/11; K/L = 40/26	–
18	38/F	Abdomen	LCD	HVV	–	–	JH–, TCRB–	CD3/19 = 62/36; CD4/8 = 28/8; K/L = 33/25	–
19	42/M	Abdomen	LCD	HVV	–	–	ND	ND	–
20	39/M	Neck	LCD	HVV	–	–	ND	ND	–

+, Positive; –, negative; IHC, immunohistochemistry; ND, not done; LLCR, λ light chain restriction; LCD, localized Castleman's disease; MCD, multicentric Castleman's disease; HVV, hyaline-vascular variant; PCS, plasma cell variant; SB, Southern blot; I, inconclusive.
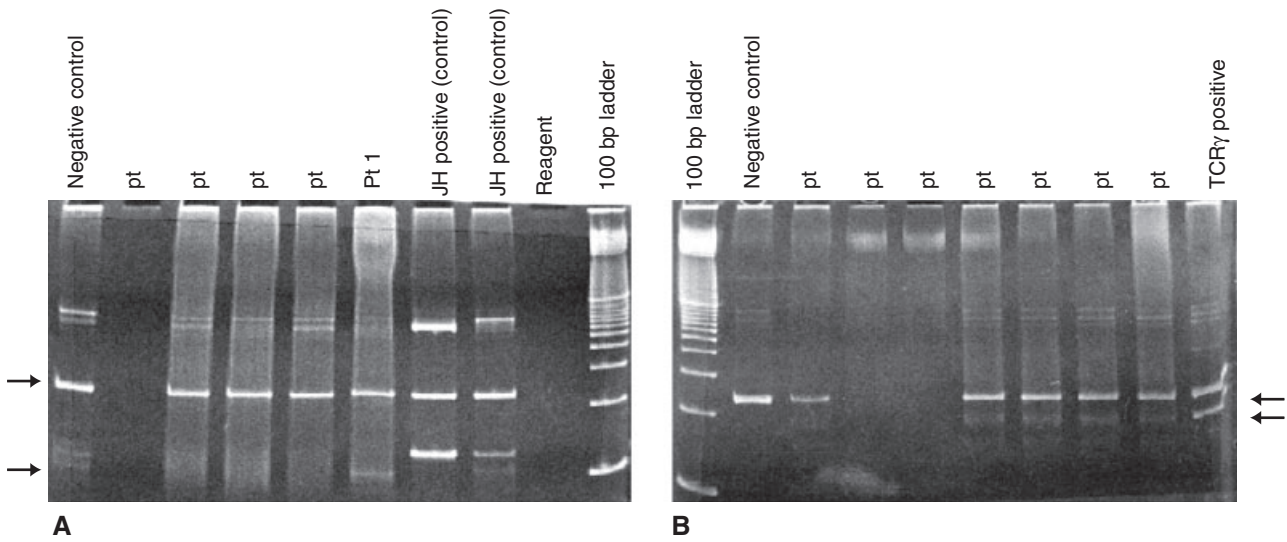
Figure 2. **A,** B-cell-specific polymerase chain reaction (PCR) using primers directed at the framework 256 (FR256) regions of the immunoglobulin heavy-chain gene (IgH). The top arrow represents the internal control that was used to ensure the presence of amplifiable DNA in each sample. Patient 1 shows the presence of a clonally rearranged IgH gene using the FR256 primers. **B,** PCR for TCRγ reveals no clonality in any of the patients.
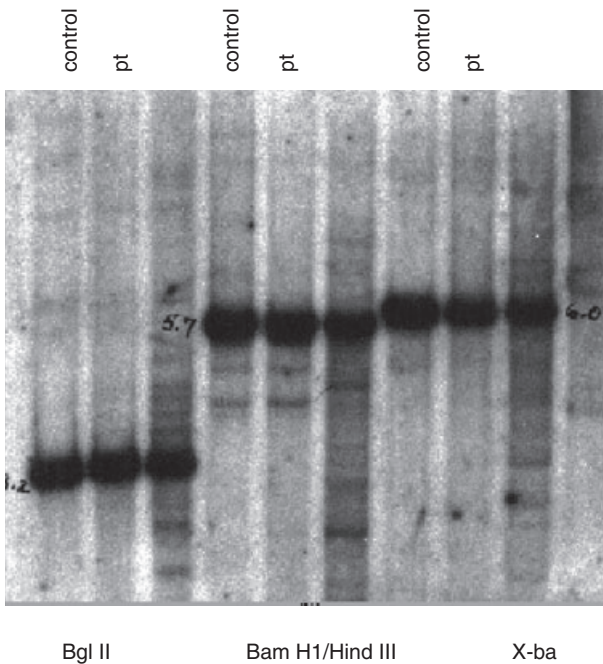
Figure 3. Southern blotting: DNA was digested using BglII, Bam HI/HindIII and XbaI for the immunoglobulin heavy chain gene and shows a germ-line pattern (patient 14).

of cases of CD the lymphocyte populations are polyclonal. Our results are similar to those of Soulier *et al.* B-cell monoclonality was detected in only one of 17 cases of LCD and in none of three cases of MCD. T-cell monoclonality was not detected in any of the 20 cases examined. IHC for κ and λ in that monoclonal case (patient 1) was not conclusive because of staining background. This case was a 65-year-old male who presented with enlarged paratracheal lymph nodes. There was no other lymphadenopathy or any systemic symptoms. All of our cases were HIV–. These findings suggest that the large majority of cases of CD do not show lymphocyte monoclonality and therefore are more in keeping with reactive than neoplastic processes.

Several investigators have examined CD for cytoplasmic immunoglobulin expression by IHC and have reported both polyclonal and monoclonal findings.[4,9,10,13–16] Our IHC results showed cytoplasmic light chain restriction in only one (patient 10) of 20 cases. However, monoclonality was not detected in that case by PCR. That case was an elderly lady, who presented with cervical lymphadenopathy and was found to have a localized CD with pathological features of the plasma cell variant. IHC was not conclusive in the case which revealed evidence of monoclonality by PCR (patient 1).

Recently, it has been suggested that CD, particularly the plasma cell and the mixed types, may be related to an excess of interleukin (IL)-6-like activity. Kaposi's sarcoma-associated herpes virus (KSHV or HHV8) encodes a functional cytokine (vIL-6) and has been found in some patients with CD.[17–21] Leger-Ravet *et al.*[21] found variable expression of IL-6 in CD and

suggested that this finding, combined with the heterogeneity of the clinicopathological presentation, indicated that different immune mechanisms are probably responsible for the different forms of the disease.

Our results indicate that the lymphoid component in CD is most commonly reactive. However, rare cases may contain a detectable monoclonal lymphoid population, and this suggests the possible outgrowth of a neoplastic lymphoid clone. Patients who show evidence of lymphocyte monoclonality probably should be followed closely for the possible development of malignant lymphoma. However, when present, it may be associated with progression to monoclonality and these cases should also be followed more closely. The biological significance of monoclonality in CD needs to be evaluated in prospective studies.

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