Lymphoid Tissues from Patients with Infectious Mononucleosis Lack Monoclonal B and T Cells

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In typical cases of infectious mononucleosis (IM), lymphoid tissue is rarely submitted for pathological examination. When lymphoid tissues from IM cases are examined, the histological appearance of IM may be difficult to distinguish from malignant lymphoma. The purpose of this study was to address the utility of clinical molecular assays for T and B cell clonality in distinguishing IM from lymphoid malignancy. DNA was recovered from paraffin-embedded archival lymphoid tissues of 18 cases of IM and 13 control cases representing other reactive lymphoid hyperplasias. T cell receptor γ (TCR- γ) and immunoglobulin heavy chain (IgH) gene rearrangements were assayed using our standard clinical polymerase chain reaction procedures targeting each of the four functional variable (V) families and the three joining (J) families of the TCR- γ gene, and framework III of the IgH gene, respectively. In 17 of 18 cases of IM, no monoclonal T or B cell populations were detectable. One case, the only spleen specimen in the study, had an oligoclonal pattern of TCR- γ rearrangements. The control cases representing other reactive hyperplasias also lacked monoclonality. The assays used were sensitive to clonal populations as small as 5% of cells. In this case series, no monoclonal lymphoid populations were identified in any case of IM. This finding suggests that molecular studies are useful in distinguishing IM from lymphoid neoplasms. (*J Mol Diag* 2002, 4:37–43)

Lymphoid tissues are rarely biopsied in infectious mononucleosis (IM) patients unless the clinical course is atypical, possibly bringing lymphoma into the differential diagnosis. In our experience, features that may provoke biopsy include: the patient's being unusually old or young for symptomatic primary Epstein-Barr virus (EBV) infection, an unusually prolonged or severe clinical course, a negative heterophile antibody test, or insufficient numbers of circulating reactive lymphocytes to suggest a

diagnosis of IM. Recognition by the pathologist of histology consistent with IM combined with a positive heterophile antibody test or EBV-specific serologies resolves the question in many cases. The presence of EBV-encoded RNA-1 (EBER1) transcripts in the tissue can confirm a diagnosis of IM, but may not necessarily exclude a malignant lymphoproliferative disorder. The causative agent of IM, EBV, is associated with about 40% of all T cell lymphomas, 5% of B cell lymphomas, most immunodeficiency-related B cell lymphoproliferations, and about 40% of Hodgkin's lymphomas.^{1–7} Lymphoid tissues from patients with IM often have histological features worrisome for non-Hodgkin's lymphoma or Hodgkin's disease.⁸ It might be useful to employ polymerase chain reaction (PCR) assays for T and B cell clonality as an adjunct to arriving at a correct diagnosis, but the expected results of clonality studies in tissues from IM patients have not been well defined. We were interested in whether or not tissues from patients with IM contain B or T cell clones as detected by routine clinical IgH and TCR- γ PCR assays for gene rearrangement. Using our routine clinical laboratory PCR assays for TCR- γ and IgH gene rearrangements, we retrospectively assayed for T and B cell clonality in paraffin-embedded tissues from a series of patients with well-documented IM.

Materials and Methods

Case Selection and Controls

Formalin-fixed, paraffin-embedded lymphoid tissues from 20 patients with IM were collected from the tissue archives of the hematopathology consultation services of two institutions, the University of Texas Health Science Center San Antonio and the University of Michigan. The cases are listed in Table 1. The patients included 18 males and two females ranging in age from 15 months to 80 years. Eleven of the patients were between 15 and 26 years old. The tissues included nine tonsils, nine lymph nodes, one nasopharyngeal mass, and one spleen. The

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Table 1. Clinical Impression, Serology, and Histopathological Diagnosis in 20 Cases of IM

Case no.	Age/gender	Clinical impression	Serological evidence of IM	Tissue type and histological diagnosis
1	25 y/male	IM, airway obstruction	Heterophile Ab positive	Tonsil: atypical reactive lymphoid hyperplasia consistent with IM
2	15 m/male	IM, lymphadenopathy	Heterophile Ab and specific EBV serologies positive	Cervical lymph node: reactive pleomorphic immunoblastic proliferation with a predominance of T cells consistent with EBV infection
3	80 y/male	Pharyngitis, no suspicion of IM	Heterophile Ab positive	Tonsil: reactive lymphoid hyperplasia consistent with EBV infection
4	17 y/female	IM	Heterophile Ab positive	Tonsil: reactive immunoblastic proliferation consistent with IM
5	26 y/male	Lymphadenopathy	Heterophile Ab positive	Cervical lymph node: atypical immunoblastic and plasmacytic hyperplasia consistent with acute EBV infection
6	32 y/male	Airway obstruction	Specific EBV serologies positive	Tonsil: acute EBV tonsillitis
7	16 y/female	Airway obstruction	Specific EBV serologies positive	Tonsil and minor salivary gland: reactive immunoblastic proliferation consistent with IM
8	38 y/male	Nasopharyngeal mass	Heterophile Ab positive and specific EBV serologies positive	Nasopharyngeal mass: atypical immunoblastic proliferation suggestive of IM
9	17 y/male	Cervical and inguinal lymphadenopathy	Heterophile Ab positive	Cervical lymph node: intense paracortical hyperplasia consistent with IM
10	21 m/male	Airway obstruction	Specific EBV serologies positive	Tonsils and adenoids: paracortical expansion with immunoblastic proliferation consistent with IM
11	50 y/male	IM, splenic rupture	Specific EBV serologies positive	Cervical lymph node: necrotizing lymphadenitis, probably infectious, with cells exhibiting large lobulated nuclei
12	5 y/male	IM, enlarged tonsils & adenoids, sleep apnea	Specific EBV serologies positive	Tonsil: atypical lymphoid proliferation consistent with IM
13	23 y/male	Lymphadenopathy	Heterophile Ab and specific EBV serologies positive	Inguinal lymph node: reactive lymphadenitis consistent with IM
14	41 y/male	Lymphadenopathy	Heterophile Ab and specific EBV serologies positive	Lymph node: lymphoid tissue with reactive paracortical hyperplasia consistent with IM
15	19 y/male	IM, splenic rupture	Specific EBV serologies positive	Spleen: benign reactive lymphoproliferation with increased lymphoplasmacellular elements within the red pulp
16	19 y/male	6 months symmetrically enlarged tonsils "atypical for IM"	Specific EBV serologies positive	Tonsil: reactive follicular hyperplasia with foci of necrosis. Adjacent are lymphoid cells with pleomorphic multilobated nuclei and immunoblasts. Favor IM.
17	17 y/male	IM	Heterophile Ab positive	Posterior cervical lymph node: atypical immunoblastic proliferation. Suggest monospot.
18	3 y/male	IM vs lymphoma	Specific EBV serologies positive	Lymph node from right neck: follicular hyperplasia with paracortical immunoblastic proliferation consistent with IM
19	15 y/male	Lymphadenopathy	Heterophile Ab positive	Lymph node: highly atypical immunoblastic proliferation
20	20 y/male	Tonsillar enlargement	Heterophile Ab and specific EBV serologies positive	Tonsils: reactive lymphoid hyperplasia with marked interfollicular hyperplasia supportive of viral etiology

diagnosis of IM had been established in these cases before inclusion in the study and was based on clinical history and serology, including report of the presence of IgM heterophile antibodies or specific EBV serologies indicative of primary EBV infection, and histopathological features of IM including EBV-encoded RNA *in situ* hybridization (EBER-ISH) showing EBV in significant numbers of

lymphoid cells. In one case (case 12) EBER-ISH could not be evaluated because of inadequate RNA preservation, but EBV latent membrane protein-1 (LMP-1) immunohistochemistry revealed positive cytoplasmic and membrane staining in a substantial fraction of lymphoid cells, verifying the EBV-related nature of the lymphoproliferation.

Table 2. Primer Sequences

Gene amplified	Region	Primer sequence	Reference no.
Immunoglobulin Heavy chain T cell receptor γ	FRIIIa	ACACGGCC(T/C)GT(A/G)TATTACTGT	11
	JHa	ACCTGAGGAGACGGTGACC	
	V1-8 consensus	TCTGG(A/G)GTCTATTACTGTGCCACCT	12
	V9	GAGGTGGATAGGATACCTGAAACGT	
	V10	CAAGTCCGTAGAGAAAGAAGACATG	
	V11	GAGAAAGAAGATGAGGTGGTGTACC	
	JP	TAATGATAAGCTTTGTTCCGGGACC	
	J1,J2 consensus	TACCTGTGACAAC(A/C)AGTGTGTTC	
	JP1,JP2 consensus	GAAGTTACTATGAGC(C/T)TAGTCCCTT	
β -Globin control	PCO3	ACACAACTGTGTTCACTAGC	13
	β GII	GTCTCCTTAAACCTGTCTTG	

Eight of these cases were reported in a previous study.⁹ One patient (case 17) had a history of Hodgkin's lymphoma diagnosed three years earlier. The original tumor tissue was not available for EBER testing nor was EBER testing performed at the time of the original diagnosis of Hodgkin's lymphoma.

A non-IM, reactive control cohort consisted of thirteen paraffin-embedded lymphoid tissue specimens (eight tonsils, four lymph nodes, and one nasopharyngeal mass), all with follicular hyperplasia as the dominant histological feature. These specimens lacked EBER positivity by ISH. Parallel ISH assays showed that U6 control transcripts were preserved and available for hybridization in all thirteen cases, demonstrating that RNA was preserved in the tissues and available for hybridization. The patients ranged in age from 14 to 61 years and included six females and seven males.

Two monoclonal control cohorts consisted of five paraffin-embedded tissues (one lymph node, one gall bladder, two skin, and one bone marrow) that were morphologically consistent with T cell lymphoma and had been proven to contain clonal T cell lymphoproliferations by Southern blot for TCR- β chain gene rearrangement and ten paraffin-embedded tissues that had been previously diagnosed as B cell lymphomas by morphology combined with immunohistochemistry and/or flow cytometry. The TCR- γ PCR assay detected monoclonal populations in four of the five T cell malignancies. The IgH PCR assay detected monoclonal IgH gene rearrangements in seven of the ten B cell lymphomas.

EBER-ISH and LMP-1 Immunostain

In situ hybridization was performed using digoxigenin-labeled riboprobes targeting EBER1 and U6 control RNA, as previously described.¹⁰ Antisense EBER1 probe is complementary to EBER1 transcripts present in latently infected cells. Detection of U6 in the target tissues signifies that RNA is preserved and available for hybridization.

For LMP-1 detection, paraffin sections were treated with 1 mg/ml pronase E (Sigma Chemical Co., St. Louis, MO) at 37°C for 5 minutes. Endogenous peroxidase was quenched using 0.1% sodium azide, 3% hydrogen peroxide in phosphate-buffered saline (PBS) for 15 minutes. A cocktail of antibodies against LMP-1 (CS1-4; Dako, Glostrup Denmark) was diluted 1:50 in autobuffer and

applied for 1 hour, followed by biotinylated rabbit anti-mouse secondary antibody, and DAB color reaction. Specific staining for LMP-1 is cytoplasmic and membranous.

Extraction of DNA from Paraffin-Embedded Tissues

Five sections 5 to 10 μ m thick were cut from each paraffin-embedded tissue block using precautions to prevent DNA carryover between samples. The sections were placed in a microfuge tube and deparaffinized by rinsing three times with xylene followed by ethanol. Dried samples were then resuspended in TEN buffer (10 mmol/L Tris-HCl, pH 8.0; 1 mmol/L EDTA, pH 8.0; and 20 mmol/L NaCl) containing proteinase K (200 μ g/ml) and incubated overnight at 55°C. Proteinase K was then inactivated at 95°C for 10 minutes, and the tissue was pelleted by full speed microcentrifugation. The supernatant containing extracted DNA was transferred to a clean tube. One microliter and 0.1 μ l quantities of supernatant were used as template in PCR assays.

Amplification of the Rearranged IgH Gene

Our routine clinical IgH rearrangement assay was used. It employs previously published primers targeting the framework 3 VH region and JH consensus sequences (Table 2).¹¹ Each sample was run twice at different template dilutions (1 μ l and 1 μ l of a 1:10 dilution in saline). Template DNA was added to a 50- μ l reaction mixture consisting of: 0.6 units AmpliTaq gold DNA polymerase (Applied Biosystems, Foster City, CA), 1X PCR buffer (Gibco Invitrogen, Carlsbad, CA), 2 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs (Applied Biosystems), and 1.0 μ mol/L of FRIII and JHa primers. The PCR reaction was run in a Perkin Elmer GeneAmp PCR system 9700 thermal cycler programmed as follows: 10 minutes at 95°C followed by 45 cycles of 95°C for 45 seconds, 55°C for 60 seconds, 72°C for 60 seconds; and ending with 72°C for 7 minutes. The following controls were included in each run: positive control (Raji Burkitt lymphoma cell line diluted 1:20 with tonsil DNA), negative control (DNA from a tonsillitis case), and a "blank" containing all of the reagents and no template DNA. The PCR products were electrophoresed in a

3.5% 2.5:1 NuSieve GTG agarose (BMA, Rockland, ME): Ultrapure DNA grade agarose (BioRad, Hercules, CA) gel in 0.5X TBE buffer containing 0.5 $\mu\text{g}/\text{ml}$ ethidium bromide at 80 V for 2.5 hours and photographed using ultraviolet illumination.

Amplification of the Rearranged TCR- γ Gene

Our routine clinical TCR γ rearrangement assay was also applied to these samples. It is a multiplex PCR which employs previously published primers targeting the V1–8, V9, V10, V11, JP, JP1,2, and J1,2 regions of the gene (see Table 2).¹² Each sample was run twice at different template dilutions (1 μl and 1 μl of a 1:10 dilution in saline). Template DNA was added to a 50- μl reaction mixture consisting of: 1.25 units of AmpliTaq gold DNA polymerase (Applied Biosystems), 1X PCR buffer (Gibco), 2.5 mmol/L MgCl_2 , 0.2 mmol/L dNTPs (Applied Biosystems), and 1.0 $\mu\text{mol}/\text{L}$ each of the seven primers (V1–8 consensus, V9, V10, V11, JP, J1/2, and JP1/2). The PCR reaction was run in a Perkin Elmer Gene Amp system 9700 thermal cycler programmed as follows: 10 minutes at 95°C followed by 40 cycles of 95°C for 60 seconds; 55°C for 60 seconds; 72°C for 60 seconds; and ending with 72°C for 5 minutes and then held at 4°C. The following controls were run with each batch of specimens: positive control (Jurkat cell line DNA diluted 1:20 with tonsil DNA), negative control (DNA from a tonsillitis case), and a “blank” containing all reagents and no template DNA. The PCR products were electrophoresed in 10% polyacrylamide gels (BioRad) in 1X TBE buffer at 95V for approximately 75 minutes. The gel was then soaked for 10 to 20 minutes in 200 ml of 1X TBE buffer containing 20 μl of 10 mg/ml ethidium bromide (Sigma, St. Louis, MO) and photographed using ultraviolet illumination.

Interpretation Criteria

In the IgH assay, samples containing one or two crisp bands in the 75 to 130 base pair (bp) region, with or without a smear of polyclonal bands in the background, were interpreted as positive for clonal IgH rearrangement. In the TCR- γ assay, samples containing one or two crisp bands in the 90 to 150 bp region, with or without a smear of polyclonal bands in the background, were interpreted as positive for clonal TCR- γ gene rearrangement. More than two crisp bands within the region of interest were interpreted as oligoclonal. In both assays, bands should be in identical positions in the undiluted and 1:10 dilution lanes to be interpreted as monoclonal (or oligoclonal) and to exclude false positive results that can be caused by degraded DNA.

In the two cases having neither a polyclonal smear nor a clonal band on the IgH or TCR- γ assays, β -globin PCR was used to test for the presence of amplifiable DNA. The procedure is the same as for IgH PCR with the substitution of 0.5 μl of 50 pmol/ μl primers for the PCO3 and BGII regions (Table 2).¹³ The β -globin product is 175 bp long.

Results

Twenty well-documented IM cases were studied. Characteristic histological features of IM were present on the hematoxylin and eosin (H&E) stained slides of biopsy material from the IM patients, in levels superficial and deep to the sections cut for DNA extraction. These features include: paracortical expansion containing small and medium sized lymphocytes, plasma cells and variable numbers of atypical immunoblasts some of which mimic Reed-Sternberg cells, minimal or absent follicular hyperplasia, and variable necrosis. The spleen from an IM patient who died of spontaneous splenic rupture exhibited increased lymphoplasmacellular elements in the red pulp and a reactive-appearing expansion of the white pulp, but it lacked the immunoblastic population seen in the other IM tissues. Figure 1 displays some of the histological features seen in our case series.

In situ hybridization revealed EBER1 localized to a proportion of small lymphocytes, many immunoblasts, and the vast majority of Reed-Sternberg-like giant cells, as seen in Figure 1C. The proportion of EBER-positive cells varied widely among cases and by histological region within a given case. The percentage of tissue lymphocytes exhibiting evidence of EBV infection by EBER or LMP-1 positivity met or exceeded 1% in all cases.

Eighteen of 20 IM cases and all control cases had amplifiable DNA. The two IM cases lacking amplifiable DNA in both the TCR- γ and IgH assays were confirmed to lack amplifiable DNA by β -globin PCR and were thereafter excluded from analysis. The TCR- γ assay revealed no monoclonal T cell populations to a sensitivity of 5% of cells in 17 of 18 IM cases, and the IgH PCR assay revealed no monoclonal B cell populations to a sensitivity of 5% in any of the 18 IM cases. The only spleen specimen studied (case 15) contained an oligoclonal T cell population as demonstrated by the identical patterns of multiple bands in duplicate samples. (Figure 2)

Discussion

In this case series, lymphoid tissues from 18 IM patients lacked monoclonal B and T cells. We limited our study to cases in which the morphological appearance of the lymphoid tissue combined with clinical, serological, and EBER-ISH results firmly established the diagnosis of IM and eliminated lymphoma from the differential diagnosis. We did not attempt to address other EBV-related lymphoproliferations such as chronic active EBV infection, fatal IM, or virus-associated hemophagocytic syndrome in the setting of EBV infection. Monoclonal T cells have been reported in some of these situations, and it has been suggested that these represent T cell lymphoma arising in the setting of IM.^{14,15}

We were interested in documenting the presence or absence of B or T cell clones as detected by routine clinical PCR assays. Approximately 90% of T cell lymphomas have monoclonal gene rearrangement which can be detected by TCR- γ PCR.¹⁶ Because of the small number of V regions within the TCR- γ gene, more rearrangements

Figure 1. Microscopic features seen in this case series include a proliferation of monomorphous large lymphocytes mimicking non-Hodgkin lymphoma (case 19) (**A**) and, more typically seen in IM, a paracortical expansion containing variable numbers of atypical immunoblasts as well as small lymphocytes and plasma cells (case 20) (**B**), features which mimic Hodgkin or T cell lymphoma. **C**: EBER-ISH pattern typically seen in IM. Both large Reed-Sternberg-like cells and many small lymphocytes display evidence of EBV RNA (violet color reaction). **A** and **B**: H&E stain, original magnification, $\times 200$, **C**: DAB with methyl green counterstain, original magnification, $\times 600$.

can be detected than can rearrangements of the TCR- β gene using a similarly complex PCR assay. Because of this, TCR- γ PCR is the more widely used test. In our laboratory we use a TCR- γ PCR assay as our routine assay for T cell clonality, and the same assay was used in this study. Similarly, we used our routine IgH PCR assay for this study. It targets the framework 3 region of the IgH

Figure 2. Lymphoid tissues from patients with IM displayed polyclonal results as shown above: IgH (**lanes 1 to 4**) and TCR- γ (**lanes 5 to 8**). **Lanes a** contain PCR products produced from undiluted template DNA. **Lanes b** contain PCR products produced from template DNA diluted 1:10. **Lane 8**, DNA extracted from the spleen (case 15), displays an oligoclonal pattern that is identical in both the undiluted and diluted specimens, eliminating concern for false positivity that may occur with degraded DNA. Neg, negative control, tonsil DNA; Pos, positive control, DNA from Jurkat (TCR- γ) or Raji (IgH) cell lines, undiluted in this photograph; Blk, blank, contains all reagents but no DNA; mw, molecular weight marker; bp, base pair.

gene and is expected to detect about 70% of B cell lymphomas. When applied to our clonal control cohorts the TCR- γ PCR assay detected clonal gene rearrangement in 80% ($N = 5$) of T cell malignancies previously shown to contain T cell clones by Southern blot analysis of the TCR- β gene. The IgH PCR assay detected clonal gene rearrangement in 70% ($N = 10$) of our control cohort of lymphomas in which B cell immunophenotype had been established by flow cytometry. Both of these assays are sensitive to clonal lymphocyte populations constituting as little as 5% of the lymphocytes in the specimen. We cannot exclude the possibility that assays with greater analytic sensitivity than ours might detect monoclonal populations comprising less than 5% of cells in the tissues we analyzed. However, we did not seek to definitively establish the presence or absence of monoclonal populations in lymphoid tissues from patients with IM, but rather to establish what the typical result would be in paraffin-embedded lymphoid tissues from patients with IM when routine clinical assays are used.

Previous reports on small numbers of lymphoid tissue specimens from IM patients have had varying results, as seen in Table 3.^{12,14,17-21} These seven studies were done on biopsied tissues from a small number of EBV-infected patients. Some patients had chronic EBV infection or IM complicated by hemophagocytic syndrome. Variable results with regard to clonality were described, with some monoclonal TCR gene rearrangements identified. Clonality assays performed on circulating lymphocytes in IM patients have also yielded varying results, including polyclonal, oligoclonal, and monoclonal T cell populations (Table 4).²²⁻²⁸ These studies used several methodologic approaches, including flow cytometry using antibodies against a selection of TCR- β chains, Southern blot and PCR directed at TCR- β and/or - γ chains, and sequencing of PCR products. Some of these studies were designed to elucidate the immunological

Table 3. Published Studies of T and B Cell Clonality in Lymphoid Tissues from Patients with IM and Variants

Reference no.	No. cases	Diagnosis	Tissue(s) studied	Molecular method(s)	Result(s) of lymphoid clonality assay(s)
17	1	IM with virus-associated hemophagocytic syndrome	Lymph node	SB: TCR- β , TCR- γ ; IgH C μ	Polyclonal
18	1	Fatal EBV infection	Lymph node and spleen	SB: TCR C β ; IgH J μ	Polyclonal
14	1	Chronic IM, abnormal T cell proliferation and hemophagocytic syndrome	Lymph node and lung	SB: TCR C β & J γ	Monoclonal during primary infection by TCR β and TCR γ assays
19	1	Atypical IM (lacked blood lymphocytosis)	Tonsil	SB: TCR C β & J β_2	Monoclonal
20	1	Chronic active EBV infection	Bone marrow	SB: TCR C β 1, J β 1, J β 2, C γ ; IgH, κ , λ	Monoclonal in TCR C β , J β 2 and C γ
21	7	IM	3 nodes, 4 tonsils, 1 nasopharynx	PCR: IgH Framework 3	Polyclonal IgH in 7/7 cases
12	3	IM	Sites not specified	PCR: TCR β , TCR- γ	1 case polyclonal, 1 case monoclonal for TCR γ only, 1 case monoclonal in TCR- β and TCR- γ

SB, Southern blot.

response to acute EBV infection and the results may not necessarily be pertinent to diagnostic hematopathology.^{24,25,27,28} The methods used in some of these studies were laborious and not suitable for routine clinical laboratory use. Only two of the referenced studies described PCR assays designed for routine clinical laboratory use. The study by Krafft et al¹² found T cell clones in 2 of 3 lymphoid tissue specimens from IM patients. The study by Short et al²⁶ found monoclonal T cells in 4 of 14 peripheral blood specimens from IM patients.

It has been suggested that the development of monoclonal or oligoclonal expansions of CD8 positive lympho-

cytes in the peripheral blood may be part of the normal response to acute EBV infection.²⁵ The blood of patients with IM has yielded evidence of oligoclonal and monoclonal populations of T lymphocytes in some studies.^{22,23,25,26,28} We did not test blood in our patients, only biopsy tissues. But, if monoclonal expansions of T lymphocytes do circulate during IM, it seems reasonable to assume that tissues heavily infiltrated by lymphocytes might yield an oligoclonal or monoclonal result. This may explain the oligoclonal T cells found in our spleen specimen (case 15). Our study suggests that these circulating T cell clones do not home to lymph nodes in significant

Table 4. Published Studies of T and B Cell Clonality in Peripheral Blood Lymphocytes from Patients with IM

Reference no.	No. cases	Diagnosis	Blood cells studied	Method(s)	Result(s)
22	8	IM	Lymphocytes	SB: TCR J β 1+2, J γ ; Ig J μ , C κ , λ probes	TCR- β : oligoclonal TCR- γ : polyclonal Ig: polyclonal
23	4	IM/chronic EBV infection	Mononuclear cells	SB: TCR C β ; Ig J μ , J κ probes	2 cases polyclonal, 2 cases monoclonal, Probes not specified
24	8	IM	CD8 ⁺ T cells	Quantitative rT-PCR using 22 TCR V β primers. Also SB in 4 cases, probes not specified	rT-PCR: all 8 cases overexpressed TCR V β 6.1-3 & V β 7 TCR. SB: 4 polyclonal
25	32	IM	CD8 ⁺ T cells	Flow cytometry using 18 V β -specific monoclonal antibodies. PCR and sequencing of TCR- β CDR3 region	Flow cytometry: 29/32 cases with particular V β expansions. Sequencing: some cases monoclonal or oligoclonal
26	14	IM	Lymphocytes	PCR for TCR- γ chain	4 monoclonal 10 polyclonal
27	1	IM	Epitope-specific CD8 ⁺ T cells	rT-PCR and sequencing of TCR- β gene, semi-quantitative PCR	Polyclonal
28	6	IM	CD4 ⁺ , CD8 ⁺ lymphocytes	Flow cytometry; PCR of TCR- β V1-24 with heteroduplex analysis	Oligoclonal CD8 ⁺ T cells

SB, Southern blot.

numbers during IM or do not survive in significant numbers in lymph nodes.

In summary, our study showed that all 18 IM tissues examined harbored neither monoclonal B nor T cell populations when assayed by routine PCR testing for IgH and TCR- γ genes. The information gained from this study may be helpful in interpreting difficult cases in which the differential diagnosis includes both IM and lymphoma. While clonality does not necessarily signify malignancy, our results indicate that the identification of a monoclonal B or T cell population by PCR assays on paraffin-embedded tissues is unusual in patients with IM and may herald a more serious process.

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