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**Oligoclonal T cells in histiocytic necrotizing lymphadenopathy are associated
with TLR9⁺ plasmacytoid dendritic cells**

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Abstract

Histiocytic necrotizing lymphadenopathy (HNL), a disease of unknown cause, is characterized pathologically by the presence of plasmacytoid dendritic cells (DCs), which are frequently mixed with oligoclonal T-cells (OTCs) and myeloid cells. Toll-like receptors (TLRs 1 to 10) are a family of pattern recognition receptors of DCs. To investigate the interactions between DCs and T cells, and to look for an etiology of HNL, we studied 24 HNLs for the profile of TLRs. Transcripts of TLR7, a receptor on plasmacytoid DCs (pDCs) for single-stranded RNA, were found in every case, confirming the universal presence of pDCs. Transcripts of TLR9, another receptor on pDCs for microbial unmethylated CpG-rich DNA, correlated with OTCs, suggesting T-cell expansion stimulated by TLR9⁺ pDCs in response to a microbe. By immunohistochemistry, we showed the pDCs were negative for the maturation marker CD83⁻. This suggested cross-priming of T-cells by immature pDCs, an immunologic concept so far based mainly on in vitro data. Transcripts of TLR5, a receptor on myeloid DCs for bacterial flagellin, correlated with abnormal blood cell counts or biochemistry. Because PCR tests for bacterial 16S rDNAs were negative in the lymph nodes, an invasive bacterium seems unlikely, but a non-invasive bacterium or a virus remains a possible candidate. Taken together, these data illustrate a novel approach, based upon TLR transcript analysis, for the integration of pathology, immunology, and clinical findings of HNL.

Introduction

Histiocytic necrotizing lymphadenopathy (HNL), also known as Kikuchi's disease, is a self-limiting lymphadenopathy with characteristic clinical and histopathologic features. The disease is especially common in young Asian females, and usually involves cervical lymph nodes with patchy subcortical necrosis and abundant nuclear debris surrounded by plasmacytoid dendritic cells (DCs) and cytotoxic T cells. Recently, myeloperoxidase-positive histiocytes were reported in HNL, suggesting the participation of monocytes or myeloid DCs. In addition, the T cells were frequently oligoclonal, implying a specific immune response in the pathogenesis of HNL. However, details of interactions between DCs and T cells, and the significance of myeloid vs lymphoid DCs, are poorly understood, partially because the etiology of HNL is still unknown.

In this report, we have developed a RT-PCR approach for characterizing the TLR repertoire. By correlating the TLR repertoire with either the clonality of T cells or the clinical manifestation, we expect to find a distinct TLR pattern that might shed light on the possible cause of HNL, clarify the details of DC-T-cell interaction, and predict the clinical outcome.

Methods and Materials

Tissue Samples

We collected 24 cases of HNL. Diagnoses were made on cervical lymph node biopsies at the time of initial presentation in the Pathology Department of the National Taiwan University Hospital between 1998 and 2001. All biopsies showed characteristic patchy subcortical necrosis and nuclear debris, with prominent infiltration of pDCs and T cells, but no granulocytes. Clinical data were obtained from the medical records. Patients with autoimmune disease or with serological or cultural evidence of bacterial or viral infections were excluded. All of the patients had a benign course, with spontaneous resolution of the lymphadenopathy within 6 months.

Analysis of Toll-like receptor transcripts by RT-PCR

The sequences of the primers for RT-PCR are listed in Table 1, which also gives the GenBank accession number of each TLR, the positions of the primers, and the sizes of the PCR products. The sequences and genomic structures of TLRs are also available in references 33-40. For example, to evaluate the TLR1 transcripts, a forward primer, 5'-ATAACAAAGGCATATTGGGCA-3' (174-194 in exon 3), and an antisense primer 5'-RS-TGTTCTTCAGATCATCTTGAT-3' (241-221 in exon 4), were used. The "RS" was a random sequence, 5'-TGACAAACTGTGTTCACTAGC-3', for increased PCR specificity and incorporation of fluorescent labels.

T-cell-receptor (TCR) γ rearrangement

4 separate nested PCRs were used for examining the 4 variable regions of *TCR- γ* , *V γ I*, *V γ II*, *V γ III*, and *V γ IV*. The primers were

V_I, 5'-TCAGGAATCAGTCCAGGAAAGTAT-3';

V_{II}, 5'-GAAAGGAATCTGGCATTCCG-3';

V_{III}, 5'-AAGCAACAAAGTGGAGGCAAGAAAG-3';

V_{IV}, 5'-CTCACACTC(T/C)CACTTC-3';

J_γ1/2, 5'-CAAGTGTTGTTCCACTGCC-3';

J_γp, 5'-TTGTTCCGGGACCAAATACC-3';

J_γp1/2, 5'-GTTACTATGAGC(C/T)TAGTC-3';

V_I' , 5'-6-FAM-TCTGG(A/G)GTCTATTACTGTGC-3';

V_{II}' , 5'-6-FAM-ATAGCTACCTACTACTGTGC-3';

V_{III}' , 5'-6-FAM-ATGGCCGTTTACTACTGTGC-3';

V_{IV}' , 5'-6-FAM-GAGGTGGTGTACCACTGTGC-3';

J_γ1/2' , 5'-AGTGTTGTTCCACTGCCAAAGAGTTT-3';

J_γp' , 5'-AGCTTTGTTCCGGGACCAAATACCTT-3';

J_γp1' , 5'-AGCTTAGTCCCTTCAGCAAATATCTT-3';

J_γp2' , 5'-AGCCTAGTCCCTTTTGCAAACGTCTT-3'.

Immunohistochemistry

Immunoperoxidase stains for, TLR9, CD123 (a marker for pDCs), CD14 (a myeloid marker), and CD83 (maturation marker of DCs) were done on formalin-fixed, paraffin-embedded tissue sections of all 24 cases. The polyclonal antibody against TLR9 was from Asia HepatoGene (Kaoshiung, Taiwan). The CD123 antibody (9F5) was from BD Pharmingen (San Diego, CA, USA). The antibodies against CD14 (NCL-CD14-223) and CD83 (1H4B) were from Novocastra (Newcastle upon Tyne, UK)

Results

1. Abundant presence of TLR7⁺ pDCs

2. Frequent oligoclonal T-cell populations in HNL

3. Strong correlation between oligoclonal T-cell expansion and the presence of TLR9⁺ pDCs

4. Immunohistochemistry (IHC) showed presence of CD123⁺ pDCs that lack the maturation marker CD83

Discussion

Kikuchi's lymphadenitis (histiocytic necrotizing lymphadenitis, HNL) is a self-limiting lymphadenopathy of unknown etiology. It is characterized by subcortical patchy necrosis with infiltrating pDCs and often oligoclonal cytotoxic T cells, but no neutrophils. Although the diagnosis is usually apparent in an endemic area like Taiwan, there are several interesting, yet unresolved questions about Kikuchi's disease. The etiology is elusive, making the diagnosis sometimes difficult in atypical cases with a protracted clinical course. Besides, data on the interaction between pDCs and T cells are limited. Finally, no prognostic factor was available for predicting the severe and protracted course in some patients.

To resolve these problems, we noticed that there are two DC subsets, pDCs and mDCs, differing in TLR expression as well as the cytokine production profile. pDCs express TLR7 and TLR9, and produce IFN- α , whereas mDCs express predominantly TLR2 and TLR4, and TLR5 and TLR8 at a lower level, and produce IL-12. In this report, we present partial answers via an extensive analysis of the TLR repertoire in the lymph nodes. We confirmed the universal presence of TLR7 pDCs, found a strong correlation between oligoclonal T-cell proliferation and the presence of TLR9⁺ pDCs, and noted that the presence of TLR5⁺ mDCs was associated with the severity of the clinical manifestations of HNL.

The correlation between oligoclonal T-cell proliferation and the presence of TLR9⁺ pDCs is consistent with reports that TLR9 is not only the most potent stimulator for cytotoxic T cells but also an essential component for cross priming. TLR9⁺ pDCs are activated by single-stranded oligodeoxynucleotides containing CpG motifs (CpG ODNs). Depending on the specific forms of

CpG ODNs, pDCs show distinct immune maturation pathways. One pathway shows limited pDC maturation but enhanced IFN- α/β secretion, resulting in the stimulation of naïve CD8 cells as well as monocyte maturation into active mDC. Our finding of TLR9⁺ immature pDCs in association with oligoclonal T-cells is consistent with this scenario.

In conclusion, by analyzing TLR repertoires, we found that TLR9⁺ pDCs correlated with oligoclonal T-cell proliferation, and TLR5⁺ mDCs correlated with severe clinical manifestations. These findings confirmed the primary role of pDCs, echoed the recent finding of cross priming by pDCs, and revealed for the first time a significant contribution of mDCs to the pathogenesis of HNL.

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計畫成果自評

Our data represent a novel model of interactions between T-cells & dendritic cells. We will use cell line model to verify these data.

Table 1. RT-PCR primers for Toll-like receptors

GenBank accession	Forward	5'-3'	Exon	Reverse	5'-3'	Exon SIZE
TLR1 AL050262	5'-ATA-ACA-AAG-GTA -TAT-TGG-GCA-3'	174- 194	3	5'-RS-TGT-TCT-TCA- GAT-CAT-CTT-GAT-3'	241- 221	4 93
TLR2 U88878	5'-GTG-GGG-CTC-ATT -GTG-CCC-ATT-3'	61- 81	2	5'-RS-CAT-CCA-CAA- AGT-ATG-TGG-CAT-3'	150- 130	3 115
TLR3 U88879	5'-AAA-AGG-AAA-GGC -TAG-CAG-TCA-3'	41- 61	1	5'-RS-AGC-ATC-CCA- AAG-GGC-AAA-AGG-3'	132- 112	2 117
TLR4 NM003266	5'-AGT-TTC-CCA-GAA -CTG-CAG-GTG-3'	513- 533	3	5'-RS-GCT-TAG-GCT- CTG-ATA-TGC-CCC-3'	593- 573	4 106
TLR5 AF051151	5'-AGT-CCC-TTC-TGC -TAG-GAC-AAC-3'	676- 696	5	5'-RS-AAG-GAA-TTC- CAA-ACA-CAG-GAC-3'	770- 750	6 120
TLR6 AB020807	5'-CAA-AAG-ACC-TAC -CGC-TGA-AAA-3'	207- 227	1	5'-RS-TGA-TAG-AAA- GCT-CAT-GTC-AGA-3'	295- 275	1 114
TLR7 AF245702	5'-TTT-GGA-AGA-AGA -CTA-AAA-ATG-3'	116- 136	2	5'-RS-AAG-GAG-TTT- GGA-AAT-TAG-GAT-3'	208- 188	3 118
TLR8 AF245703	5'-TTC-TGC-GCT-GCT -GCA-AGT-TAC-3'	1- 21	1	5'-RS-ACG-ACT-GAA- GGA-ACA-TGT-TTT-3'	73- 53	2 98
TLR9 AF245704	5'-AAG-CCC-CTG-CCC -CCC-AGC-ATG-3'	127- 147	1	5'-RS-CCA-GCA-TGA- TGG-CCT-GCA-CCA-3'	205- 185	2 104
TLR10 AF296673	5'-ATT-ATG-CTT-CTC -CTC-TCT-GAG-3'	306- 326	1	5'-RS-AGA-GCA-TTG- GCT-GAG-AAG-TCT-3'	396- 376	1 116
β_2 M	5'-CTT-TGT-CAC-AGC -CCA-AGA-TAG-3'	1589- 1609	2	5'-RS-GCA-GAA-TTT- GAA-TTC-ACT-CAA-3'	3549- 3529	4 126