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Original article

Identification and the preliminary *in vitro* characterization of IRIS homologue from salivary glands of *Ixodes persulcatus* Schulze

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ABSTRACT

Ixodes ricinus immunosuppressor (Iris) is a tick salivary gland protein derived from *I. ricinus*. In this study, Iris homolog was identified in the salivary glands of *Ixodes persulcatus*, which is the specific vector of the Lyme disease agent in Japan. The homolog was named Ipis-1. To investigate the function of Ipis-1, we prepared a recombinant Ipis-1 expressed in COS-7 cells as a rabbit IgG Fc-fused protein (Ipis-1-Ig). Cell proliferation assay and IFN-γ ELISA showed that Ipis-1-Ig inhibits the proliferation and IFN-γ production of bovine peripheral blood mononuclear cells (PBMCs). Notably, Ipis-1-Ig inhibited the cell proliferation and production of IFN-γ in bovine PBMCs even when $CD14^+$ cells were depleted, suggesting that Ipis could directly interact with T cells and inhibit their functions. In conclusion, Ipis could contribute to the establishment of environments suitable for tick blood feeding and pathogen transmission by suppressing the function of immune cells.

1. Introduction

Ixodid ticks feed on blood and are harmful vectors for several pathogens that cause infectious diseases, affecting both human public health and livestock industry (Tellam et al., 1992; Sonenshine, 1993; Charrel et al., 2004; de la Fuente and Kocan, 2006). Recent studies have focused on the development of an anti-tick vaccine that could inhibit blood feeding and oviposition of ticks. There have been several reports on the identification and characterization of tick molecules as effective targets for anti-tick vaccines (Kay and Kemp, 1994; Willadsen, 2004; de la Fuente et al., 2007).

Ixodes ricinus immunosuppressor (Iris) is a tick salivary gland protein derived from *I. ricinus*, which is the major vector of the Lyme disease agent in western Europe (Leboulle et al., 2002a). Iris inhibits the proliferation of mouse splenocytes and the production of several cytokines such as IFN-γ, IL-6, TNF-α, and IL-8 in human PBMCs (Leboulle et al., 2002b). It is also known to bind to monocytes/macrophages and inhibit their TNF-α secretion (Prevot et al., 2009). Iris is a member of the serine protease inhibitor superfamily and interferes with coagulation (Prevot et al., 2006). Thus, Iris is expected to be a new candidate antigen for anti-tick vaccines because of its multi-functional properties. Notably, Prevot and co-workers (2007) have reported that the protective immunity against ticks in Iris-vaccinated rabbits results in an increased mortality rate and a decreased weight gain in both nymph and adult ticks and an increased blood-feeding time in adult ticks. Notably, it has been proposed that Iris is a vaccinating antigen interacting with both host immunity and hemostasis (Prevot et al., 2007).

In Japan, *Ixodes persulcatus* is the specific vector of the Lyme disease agent (Nakao and Miyamoto, 1994; Murase et al., 2013). In addition to this information, *I. persulcatus* has recently drawn some attention as a vector for *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis (Ohashi et al., 2005) and tick-borne fever in several different animals (Murase et al., 2011), and *Borrelia miyamotoi*, the causative agent of relapsing fever (Taylor et al., 2013; Takano et al., 2014). Previously, we have established an *I. persulcatus* laboratory colony to understand the tick's biology and events in *I. persulcatus*-borne pathogen

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(A)

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1    TTCGGAAGTGCAATATCCTGTCATTAAGCACTAATTTTCATACCGTGAGTTGATCAGAGGCCCCTCTTAACGAAACATTAAAAAAAGGATCTAAGATGGA
                                                                                                  M  E
101  GGCAAGTCTGAGCAACCACATCCTTAACTTCTCCGTCGACCTATACAAGCAGCTGAAACCTTCCGGCAAAGACACGGCAGGAAACGTCTTCTGCTCACCA
       A  S  L  S  N  H  I  L  N  F  S  V  D  L  Y  K  Q  L  K  P  S  G  K  D  T  A  G  N  V  F  C  S  P
201  TTCAGTATTGCAGCTGCTCTGTCCATGGCCCTTGCAGGAGCTAGAGGCAACACTGCCAAGCAAATCGCTGCCATCCTGCACACAAACGACGACAAGATCC
       F  S  I  A  A  A  L  S  M  A  L  A  G  A  R  G  N  T  A  K  Q  I  A  A  I  L  H  T  N  D  D  K  I
301  ACGACCACTTCTCTAACTTCCTTAGGAAGCTACCCAGTTACGCCCCAGATGTGGCCCTGCACATCGCCAATCGCATGTACTCTGAGCAGACCTTCCATCT
       H  D  H  F  S  N  F  L  R  K  L  P  S  Y  A  P  D  V  A  L  H  I  A  N  R  M  Y  S  E  Q  T  F  H  L
401  GAAAGCGGAGTACACAACCCTGTTGCAGAAGTCCTACGACAGCACCATCAAGGCTGTTGACTTTGCAGGAAACGCCGACAGGGTCCGTCTGGAGGTCAAT
       K  A  E  Y  T  T  L  L  Q  K  S  Y  D  S  T  I  K  A  V  D  F  A  G  N  A  D  R  V  R  L  E  V  N
501  GCCTGGGTTGAGGAAGTCACAAGGTCAAAGATCAGAGACCTGCTCGCACCTGGAACTGTTGATGCATTGACATCACTTATATTGGTGAATGCCATTTACT
       A  W  V  E  E  V  T  R  S  K  I  R  D  L  L  A  P  G  T  V  D  A  L  T  S  L  I  L  V  N  A  I  Y
601  TCAAAGGTCTGTGGGAGTGTCAATTCAAGCCTAGTGCTACGAAGCCGGGAGATTTCCACTTGACGCCACAAACCTCAAAGACAGTGGACATGATGCACCA
       F  K  G  L  W  E  C  Q  F  K  P  S  A  T  K  P  G  D  F  H  L  T  P  Q  T  S  K  T  V  D  M  M  H  Q
701  GAAAGGGGACTTCAAGATGGGTCACTGCAGCGACCTCAAGGTCACTGCGCTTGAGATTCCCTACAAAGGCAACAAGACGTCGATGGTCATTCTCCTGCCC
       K  G  D  F  K  M  G  H  C  S  D  L  K  V  T  A  L  E  I  P  Y  K  G  N  K  T  S  M  V  I  L  L  P
801  AAAGATGTGGAGGGACTCTCAGTCCTGGAAGAACACTTGACCGCTCCGAAACTGTCGGCTCTGCTCGGCGGCATGTATGTGACGTCCGATGTCTACTTGC
       K  D  V  E  G  L  S  V  L  E  E  H  L  T  A  P  K  L  S  A  L  L  G  G  M  Y  V  T  S  D  V  Y  L
901  GCTTGCCGAAGTTCAAACTGGAGCAGTCCATAGGTTTGAAGGACGTACTGATGGCGATGGGGGTCAAGGATTTCTTCACATCCCTTGCAGATCTTTCTGG
       R  L  P  K  F  K  L  E  Q  S  I  G  L  K  D  V  L  M  A  M  G  V  K  D  F  F  T  S  L  A  D  L  S  G
1001 CATCAGCGCTACGGGGAATCTGTGCGCTTCGGATGTCATCCACAAGGCTTTTGTAGAAGTTAATGAGGAGGGCACAGAGGCTGCAGCTGCTAATGCCATA
       I  S  A  T  G  N  L  C  A  S  D  V  I  H  K  A  F  V  E  V  N  E  E  G  T  E  A  A  A  A  A  N  A  I
1101 CGCCTGAGGTATATGTGTTTAAGATTTCCACAGGTAATTAACTTTTTTGTTGACCGCCCATTCATGTTCTTGATCCACAGCCATGATCCAGATGTTGTTC
       R  L  R  Y  M  C  L  R  F  P  Q  V  I  N  F  F  V  D  R  P  F  M  F  L  I  H  S  H  D  P  D  V  V
1201 TCTTTATGGGATCCATCCGTGAGCTCTAAGAAGCATTGCTCTTAATGGCGACCAATCAGTCCGGAAGGTTATCTCTTAGTCACTAATTGTAACAATTCTG
       L  F  M  G  S  I  R  E  L  *
1301 CAATATTCAGCTTGTGTATTTCAGTAACTTGCTAGATCTTTGTCTTGTTGATGTTAGGCTTCTTGTGAGATGTTGTGCTTTAAAATAGAGATGGGGACTG

1401 AAGTAAATAAACGAAGTTAAAGCCATGAAAAAAAAAAAAAAAA  1443
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(B)

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Iris     MEASLSNHILNFSVDLYKRLKPSGKDTAGNVFCSPFSIAAALSMALAGARGNTAKQIAAI  60
Ipis-1   ..................Q.............................................  60

Iris     LHSNDDKIHDHFSSFLCKLPSYAPDVALHIANRMYSEQTFHPKAEYTTLLQKSYDSTIKA  120
Ipis-1   ..T.........N..R.........................L.....................  120

                                                    s3a domain
Iris     VDFAGNADRVRLEVNAWVEEVTRSKIRDLLAPGTVDSSTSLILVNAIYFKGLWDSQFKPS  180
Ipis-1   ....................................AL..............EC.....  180

Iris     ATKPGDFHLTPQTSKKVDMMHQKGDFKMGHCSDLKVTALEIPYKGNKTSMVILLPEDVEG  240
Ipis-1   ................T.....................................K....  240

Iris     LSVLEEHLTAPKLSALLGGMYVTSDVNLRLPKFKLEQSIGLKDVLMAMGVKDFFTSLADL  300
Ipis-1   ........................Y.............................  300

                                          RCL domain                Serpin signature
Iris     SGISAAGNLCASDIIHKAFVEVNEEGTEAAAATAIPIMLMCARFPQVVNFFVDRPFMFLI  360
Ipis-1   .....T.......V..........|.....N..RLRY..L.....I.|..........  360

Iris     HSHDPDVVLFMGSIREL*  378   Identity (%)
Ipis-1   ..................  378   94.4%
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Fig. 1. Cloning of Ipis from *I. persulcatus* (GenBank accession no. AB971750). (A) Nucleotide and deduced amino acid sequences of cDNA encoding for Ipis-1 from *I. persulcatus*. A poly(A) addition signal (AATAAA) is boxed. (B) Sequence alignment of Ipis with Iris from *I. ricinus* (AJ269658). The serine protease inhibitor-associated domains (s3a domain, RCL domain, and serpin signature) are boxed.

transmission (Konnai et al., 2008), and identified some molecules such as defensin (Saito et al., 2009), Salp15 (Mori et al., 2010), lipocalins (Konnai et al., 2011), TROSPA (Konnai et al., 2012), Salp16 (Hidano et al., 2014), and metalloproteases (Ali et al., 2014). However, unlike the abundant data available for *Ixodes scapularis* or *I. ricinus*, there is only limited information concerning such molecules from *I. persulcatus*. In addition, vaccinating antigens against *I. persulcatus* have not been reported till date.

In this study, to determine whether the Iris-like molecule from *I. persulcatus* inhibits the immune response, we cloned and sequenced *Iris*-like transcript from this organism and subsequently generated a recombinant protein for immunoassays.