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Immunoregulatory role for a public IgM idiotype in the induction of autoimmune diseases in *Mycoplasma pneumoniae* infection

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ABSTRACT

Mycoplasma pneumoniae (MP) infection is associated with the emergence of various autoimmune disorders and autoantibody production. The most common autoantibodies induced are of anti-I specificity and express cold agglutinin (CA) activity. However, the mechanisms by which the microbial infection triggers the appearance of these autoantibodies are still unknown. To investigate these mechanisms, we used BALB/c mice as experimental models. In this paper, we show that BALB/c mice polyclonal antisera to MP react with human CA IgMs, and reciprocally, that BALB/c mice polyclonal antisera to human IgM CA react with MP. However, antibodies directed against MP and against CA IgM triggered by both immunizations represent two separate sets of antibodies. This was also confirmed using monoclonal antibodies derived from the immunized mice. Among these MAb we selected a monoclonal antibody MAb1D3 which reveals a cross-reactive idiotope (CRI) shared by human CA and other MIgMs with various autoantibody activities (anti-MAG and anti-IF). The CRI defined by MAb1D3 is a recurrent interspecies idiotope that is expressed by post infectious IgM antibodies to MP. Hence, we present in this study new data showing that the concomitant appearance of CAs and anti-MP IgM antibodies during acute MP infection is the consequence of a common idiotypic regulation of antibodies to infectious and to self antigens.

1. Introduction

Mycoplasma pneumoniae (MP) is a prokaryote which causes primary atypical pneumonia in humans [1,2]. This infection is more commonly reported among children and young adults [3,4]. Tissue colonization by MP requires cytadherence of the microorganism to respiratory epithelial cells [5–7]. A trypsin sensitive surface protein designated P1 is likely to be the major adhesin of MP [8,9]. Ligands of P1 adhesin were identified as glycoprotein expressing I/i oligosaccharides, sialyl glycoproteins and sulfated glycolipids, present in various host cells and tissues [10,11]. The role of P1, as adhesin, is supported by the demonstration that monoclonal [12] and monospecific [13] anti-P1 antibodies inhibit MP attachment to the respiratory epithelium. Protein P1 elicits a strong immune response in humans and experimentally infected animals with MP [14], indicating it is the major immunogen. The cytadhesin P1 gene was cloned and its protein sequence was deduced [15,16]. An immunogenic epitope of 13 amino acid components (Pep13P1) involved in the pathogen cytadherence has been identified [17].

MP infection is often associated with various autoimmune disorders including autoimmune anemia, neuropathy, cardiopathy and autoantibody production [18–21]. However, the mechanisms by which the microbial infection triggers the appearance of these autoantibodies are still unknown. The most common autoantibodies induced by MP infection are of anti-I specificity and express cold agglutinin (CA) activity [22,23]. Their occurrence is sufficiently high to be of diagnostic significance. CAs, which develop during MP infection, belong to the IgM class, have anti-I antigen specificity [24] and are generally harmless to the patient, but in a few cases they may induce transient autoimmune haemolytic anemia (A.I.H.A.) [3,25].

Considerable controversy exists as to the mechanism of induction of anti-I CA in patients with MP infection and various hypotheses have been proposed. CAs might be cross-reacting antibodies, which would recognize an I-like antigen on MP [26]. Alternatively, Ten Feizi and coworkers have demonstrated that MP interacts with human erythrocytes via molecules of I-i antigen type and proposed that I-antigen becomes autoimmunogenic after interaction with MP [10]. However, some previous data of our laboratory are in agreement with a third hypothesis involving the idiotypic network. Indeed, we have shown that IgM from a patient with primary MP infection were tightly associated with IgG, most likely as immune complexes exhibiting CA and anti-MP activ-

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Table 1
MAb 1D3 reactivity with a panel of human MIgM.

HMIgM	Activity	Light chain	Subgroup VH	Subgroup VL	1D3CRI
IgM ALB	Anti-I (CA)	Kappa	ND	ND	+
IgM LAK	Anti-I (CA)	Kappa	ND	ND	+
IgM POT	Anti-I (CA)	Kappa	ND	ND	+
IgM TUR	Anti-i (CA)	Kappa	ND	ND	+
IgM MOU	Anti-Pr (CA)	Kappa	ND	ND	+
E4HMF9 IgM	Anti-I (CA)	Kappa	ND	ND	+
IgM TOR	Anti-Pep13P1	Kappa	ND	ND	+
IgM GEN	Anti-Pep13P1	Kappa	ND	ND	+
IgM AZI	Anti-MAG/Pep13P1	Lambda	VH II	VLamda II	+
IgM YSE	Anti-MAG/Pep13P1	Kappa	VH III,JH4	VKappa IIIa	+
IgM BOU	Anti-MAG	Kappa	VH III	VKappa I	+
IgM ROG	Anti-MAG	Kappa	VHIII	VKappa III	+
IgM MOR	Anti-MAG	Kappa	ND	ND	-
IgM MON	Anti-MAG	Kappa	ND	ND	-
IgM DEP	Anti-MAG	Kappa	VHIII,JH4	VKappa IV	-
IgM GON	Anti-Vimentin/Pep13P1	Kappa	ND	ND	+
IgM DUV	Anti-Vimentin	Kappa	ND	ND	-
IgM HUC	Anti-Sm	Lambda	ND	ND	-
IgM MAR	Polyreactive	Lambda	VH4-18	ND	-
IgM MAL	Polyreactive	Lambda	VH4-18	ND	-
IgM IPT	Unknown	Kappa	ND	ND	-
IgM HAT	Unknown	Kappa	ND	ND	-
IgM GLU	Unknown	Kappa	ND	ND	-
IgM LOU	Unknown	Kappa	ND	ND	-
IgM BOI	Unknown	Lambda	ND	ND	-

Panel of human monoclonal IgMs. Data shows structural characteristics, antibody specificities and MAb 1D3 reactivity of the human MIgMs used in this study.

ity [27]. On the other hand, we have shown that polyclonal IgMs secreted by cord blood B cells, which represent the early immune repertoire and are mainly composed of natural antibodies, react with Pep13P1 [28]. All these data suggest that IgM anti-Pep13P1 induced by MP infection might be functionally related to the repertoire of natural antibodies, including natural autoantibodies proven to be strongly regulated via the idiotypic network [29]. In this study we present new data showing that the concomitant appearance of CA and anti-P1 antibodies during acute MP infection is the consequence of an idiotypic network dysregulation of the IgM autoreactive repertoire.

2. Materials and methods

2.1. Patient LK

Child LK, 6 years old, was admitted for a primary atypical pneumonia with A.I.H.A. A Direct Coombs' test was positive for complement and was associated with a transient rise in the serum titer of CA of anti-I specificity, which peaked at 1:16000 on day 12 and returned to normal levels within 6 weeks. Serology for MP was positive for IgM and IgG antibodies as assessed by ELISA on MP or synthetic peptide P1 (Pep13P1) coated plates. The IgM titer was the highest (1:4000) at the peak of CA activity and declined to 1:128 within 6 weeks. On admission, electrophoresis of serum protein revealed a monoclonal component identified by immunoelectrophoresis as IgMκ. The restricted heterogeneity of IgM LK was confirmed by isoelectric focusing. The M component regressed spontaneously over the first 6 weeks and was undetectable 5 months later. Purification of CAs from serum LK by fixation elution on OI+Rh negative red blood cells showed that they corresponded to the IgMκ component. IgM LK fraction did not contain any rheumatoid factor activity as assessed by ELISA on human IgG coated plates [27].

2.2. Sera and monoclonal human IgMs

A panel of 20 human monoclonal IgMs (MIgMs) from patient sera with lymphoproliferative diseases was used in this study, (Table 1). 5 CA IgMs were from patients with chronic cold agglutinin disease (C.C.A.D) [30]. 15 MIgMs were purified from patient's sera with Waldenström macroglobulinemia (W.M): 4 were with unknown antibody activity and 11 showed antibody reactivity to vimentin intermediate filament protein (IF) [31] or to myelin-associated glycoprotein (MAG) [32] and/or to Pep13P1 peptide of MP [33]. Among these, 5 IgMs have been analyzed for their nucleotidic sequence of the variable domains [34–37].

Anti-MAG IgM YSE expressing anti-Pep13P1 activity was also available as a supernatant of a clone derived from EBV transformed lymphoblastoid B cell line of patient YSE and was kindly provided by Pr J.C. Brouet (Hôpital Saint Louis, Paris).

Supernatant of a mouse–human heterohybridoma E4HMF9 secreting a human IgM with anti-I CA activity was kindly provided by Lena EDELMAN (Institut Pasteur de Paris, France).

Five supernatants of B cell lines were kindly provided by Pr Guillaume Dighiero (Institut Pasteur de Paris), 2 containing a polyreactive IgM λ, 1 an IgM λ anti-Sm, and 2 an IgM λ with unknown specificity (Table 1).

2.3. Mycoplasma antigen

The MP strain used in this study is the FHATCC No. 15531 strain, maintained in culture at the Mycoplasma laboratory of the Institut Pasteur de Tunis. MP was collected by centrifugation at 12000 × g, washed three times in saline, and used for ELISA experiments or mice immunization.

Pep13P1 peptide was kindly prepared by M. Bahraoui and K. Mabrouk (Laboratoire de Biochimie, Faculté de Médecine de Nord-Marseille, France). The Pep13P1 peptide sequence is Gly–Ile–Val–Arg–Thr–Pro–Leu–Ala–Glu–Leu–Leu–Asp–Gly.

2.4. Purification of human IgMs and IgGs

Human MIgMs and polyclonal IgM and IgG were purified from patients or healthy adults sera respectively by precipitation with 40% saturated ammonium sulfate, followed by Sepharose 6B chromatography. The first peak was dialyzed against 0.0175 M, phosphate buffer saline (PBS) pH 7.6 and passed over a DEAE-