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Anti-polysaccharide IgA seen to be more polyreactive than IgG antibodies

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Abstract

Polyreactive antibodies are natural antibodies. They bind with a low affinity to a variety of structurally unrelated antigens. IgA is the main antibody isotype that protects mucosal surfaces and can be produced against thymus-independent (polysaccharide) or thymus-dependent antigens (proteins). Nevertheless, the mucosal IgA polyreactive responses to polysaccharides are unknown. Neisseria meningitidis and Salmonella Typhi Ps were selected as the mucosal microorganisms. To explore the predominance of IgA over IgG isotypes in polyreactivity, a hybridoma system induced by polysaccharides was used. Mice were immunized with a standard protocol to produce monoclonal anti-Ps C from N. meningitidis and the Vi of S. Typhi antibodies. We used non-specific hybridoma supernatants to detect IgG and IgA anti A, C, Y, X, and Vi polyreactivity by indirect ELISA. Only one monoclonal antibody of nine secreting hybridomas in each system was obtained. Polyreactive responses against polysaccharides A, C, Y, and X of N. meningitidis and Vi of S. Typhi were obtained mainly from the IgA isotype. Only one polyreactive IgG was obtained from each system. Polyreactive anti-polysaccharide IgA responses predominate during hybridoma production and were found for the first time. The selection of polysaccharides as antigens for mucosal immunization could be a strategy to induce a polyreactive response.

Keywords: polyreactivity, IgA, polysaccharide, monoclonal antibodies

Introduction

Polysaccharides (Ps) have a repetitive structure; they are thymus-independent antigens that are capable of directly activating B lymphocytes without T lymphocyte cooperation, and the antibody results of this stimulation produce low-affinity antibodies [1,2]. The IgA response can be induced in thymus-dependent and -independent manners [3]. Many microorganisms have Ps in their structure and are virulence factors. Neisseria sp. is a human mucosal obligate pathogen or non-pathogenic species, and N. meningitidis has 12 serogroups. This classification is based on Ps capsular expression. The A, B, C, W, X, and Y serogroups were the most disease-causing agents. S. Typhi belongs to the genus Salmonella, which has only Vi Ps as a capsular antigen and colonizes mucosal surfaces (intestine). N. meningitidis and S. Typhi infect mucosal surfaces where IgA is the main protecting antibody; however, systemic vaccinations are currently used that do not induce mucosal responses. At present, antigen delivery to mucosal surfaces is very interesting, especially for protection against infectious agents that are mediated mainly by the local immune response. However, mucosal immunization has been limited by important aspects such as low immunogenicity [4], mainly due to the absence of mucosal adjuvant [5,6].

In 1975, Kohler and Milstein devised a revolutionary method for preparing monoclonal antibodies (mAbs) that are target-specific against a single antigen [7]. The cell biology technology permits the fusion of a B-cell with a myeloma cell to form a “hybrid’ cell with the properties of both parental cell lines. This fusion gives rise to a cell called hybridoma, which produces antibodies with a single specificity known as mAb. Therefore, most research is focused on specific mAb production, and all other hybridomas are discarded. Therefore, we decided to evaluate the polyreactivity of hybridomas induced by Ps immunization.

Polyreactivity is a conserved feature of antibodies among species and can be found in IgM, IgG, and IgA isotypes [8]. These antibodies can bind to a variety of structurally unrelated self and non-self-antigens [9] and generally have low affinity [10]. Polyreactivity antibodies are a major component of the natural antibody repertoire and bind with low affinity to a variety of structurally unrelated antigens. The union between polyreactive antibodies and epitopes is not a rigid structure, but a flexible antigen- binding pocket that can accommodate antigens that bind to different residues [11]. The conformational changes and clonal selection that are evidenced can be seen as complementary processes that increase the diversity of antibodies and are not mutually exclusive [12]. In our lab, we are working with the polyreactivity induced by Ps, using potent adjuvants such as cochleate and mucosal route of vaccination [6,7,13].

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To explore the predominance of IgA over IgG isotypes in polyreactivity, the hybridoma system induced by Ps was used.

Materials and Methods

Polysaccharides and hybridoma of anti C polysaccharide of N. meningitidis

Capsular Ps of Neisseria meningitidis A, C, Y, and X serogroups were obtained from Finlay Institute production plants under good manufacturing practices. Monoclonal antibodies were obtained using the traditional methodology at the Monoclonal Department of the Finlay Institute [14]. Briefly, BALB/c mice were immunized intraperitoneally with Ps from N. meningitidis serogroup C or Vi of S. Typhi in an appropriate adjuvant. After 3 weeks, antibody titers were measured by ELISA, and B cells were obtained from the spleen and fused with myeloma cells. Hybridomas were screened and cloned to produce specific antibodies. Specific anti-Ps antibodies were obtained from the IgG class. All discharge nonspecific hybridoma-producing clones were evaluated for the recognition of Ps polyreactivity.

All experiments were performed with the approval of the Ethical Committees for Laboratory Animals at the Finlay Institute.

Analysis of culture supernatant of hybridomas antibody Response by ELISA

Maxisorp 96-well plates (Nunc) were coated with 100 µL of N. meningitidis polysaccharide A, C, Y, or X or Vi Ps from S. Typhi (10 µg/mL) in 0.05 M carbonate buffer (pH 9.6) for 4 h at room temperature, followed by overnight incubation at 4°C. The plates were blocked with 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 min at 37 °C. The dilutions of the culture supernatants were incubated for 1 h at 37°C. After washing with 0.05% Tween-20, the plates were incubated for 1 h at 37 °C with goat anti-mouse IgG (Southern Biotechnology Associates, Inc., Birmingham, AL), or anti-mouse IgA (Southern Biotechnology Associates, Inc., Birmingham, AL) coupled to horseradish peroxidase in 1% BSA-PBS buffer. The plates were then washed with 0.05% Tween-20 and developed using 100 µL of 1 mg/mL o-phenylenediamine dihydrochloride (Sigma) in 0.1 M citrate buffer (pH 4.5) in the presence of 0.04% H2O2. The reaction was stopped by the addition of H2SO4. The absorbance was read at 492 nm and expressed as the optical density (OD).

Mice were euthanized by cervical dislocation. The spleen was removed and macerated. Osmotic shock with ammonium chloride was necessary to remove erythrocytes and obtain lymphocytes. All experiments were performed with the approval of the Ethical Committees for Laboratory Animals at the Finlay Institute.

Results

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Nine productive hybridomas were obtained against the CPs of N. meningitidis. One productive hybridoma recognized only the Ps C of the IgG class and was selected as the mAb. The other eight were Ps C non-specific productive hybridomas. Seven (87.5%) were IgA isotypes and only one (12.5%) was IgG isotype. The polyreactive response against N. meningitidis Ps of the main serogroups A, C, Y, or X was evaluated. All hybridomas recognize Ps C as an inductive immunogen. IgG hybridoma recognizes A, C, and X Ps. The IgA hybridomas recognized: Ps A (five hybridomas, 71.4%), Y (five hybridomas, 71.4%), and X (five hybridomas, 71.4%) from N. meningitidis, in addition to C Ps, were observed (Table 1).

Table 1. Polyreactive response against different serogroups of Neisseria meningitidis induced by serogroup C polysaccharide. A, C, Y, and X are polysaccharides of N. meningitidis serogroups; mAb, monoclonal antibody; + clones with polyreactive response; -, no response.

Class	*Neisseria meningitidis* polysaccharides				Observation
	C	A	Y	X	mAb
IgG	+	-	-	-	
IgG	+	+	-	+	Polyreactive
IgA	+	+	+	+	
	+	+	+	-	
	+	+	+	+	
	+	-	+	-	
	+	-	-	+	
	+	+	+	+	
	+	+	-	+	

Similar results were obtained when IgA and IgG responses were analyzed in the culture supernatant of hybridomas induced by immunization with the Vi of S. Typhi immunogen. As expected, all hybridomas recognized Vi Ps, and one of the IgG classes only recognized Vi and was selected as the mAb. One IgG was polyreactive against A and Vi (12.5 %), and seven IgA were polyreactive against Y and Vi (87.5 %), of which one recognized PsA (Table 2).

Table 2. Polyreactive responses against different serogroups of Neisseria meningitides induced by Vi polysaccharide of Salmonella Typhi. A, C, Y, and X are polysaccharides of N. meningitidis serogroups; mAb, monoclonal antibody; (+) clones

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with polyreactive response; (-) clones with no response.

Class	Polysaccharides				Observation
	Salmonella Typhi	*Neisseria meningitidis*			
	Vi	A	C	Y	mAb
IgG	+	-	-	-	Polyreactive
IgG	+	+	-	-	
IgA	+	+	-	+	
	+	-	-	+	
	+	-	-	+	
	+	-	-	+	
	+	-	-	+	
	+	-	-	+	
	+	-	-	+	

Discussion

Polyreactive antibodies against polysaccharides of the mucosal pathogens N. meningitidis and S. Typhi induced by C or Vi Ps as immunogens during monoclonal production were obtained. All hybridomas recognized inducer immunogens. The IgA polyreactive isotype was predominant against most immunogens.

Immunization of the host with a purified antigen results in the development of high-affinity antibodies that react with immunizing immunogens. However, for more than 100 years, normal serum is known to contain low-affinity antibodies that react with self-antigens and some foreign antigens to which the host has never been exposed [15]. These antibodies are known as natural antibodies.

At present, it is well known that these antibodies like “natural antibodies,” coming from innate response and are mainly of IgG, IgA, and IgM classes. A subgroup of these antibodies is polyreactive antibodies that can bind with low affinity to non-related antigens [16]. Several explanations have been proposed, one of which is the union site of these antibodies, where the epitope binding pocket is more flexible than specific

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antibodies and can accommodate antigens that bind to different residues within the binding pocket [11].

In our work, we obtained this type of response, since in a hybridoma panel against a single antigen response was obtained against some related antigens (even some of different types), mainly of the IgA class. If we add that IgA can also be produced with low affinity, this fact will reaffirm that IgA not only can give more cross-reactivity but also a more polyreactive response than other types.

Zhou et al. obtained a monoclonal antibody and polyreactive IgM isotype antibodies and tested the binding capacity of these antibodies against a panel of gram-negative and gram-positive bacterial antigens. Only polyreactive antibodies were able to bind indistinctly to both with different affinities [11]. Moreover, the gram-positive bacteria to which the polyreactive antibody and complement bound grew at a slower rate and were lysed and phagocytosed by macrophages [2]. The production of IgM isotypes should be addressed in future studies. In addition, the IgG2 subclass is the IgG that recognizes polysaccharides against it is the second one with a higher serum concentration.

Polyreactive antibodies bind to antigens with lower affinities as compared with monoclonal antibodies, and each polyreactive antibody has a distinct antigen-binding pattern that can vary for different antigens [18]. In our study, we obtained different grades of responses (OD) against different serogroups (results not shown). The antigen-binding pattern may be caused by the conformational changes between these polysaccharides.

Although N. meningitidis and S. Typhi infect mucosal surfaces where IgA is the main protecting antibody, systemic vaccination is currently used, which does not induce a sustained mucosal response. At present, antigen delivery to mucosal surfaces is very interesting, especially for protection against infectious agents that are mediated by local immune responses. However, mucosal immunization is limited by important aspects such as safety and immunogenicity. The results obtained suggest that polyreactive antibody functions are not completely elucidated, and further studies in this field are necessary, mainly in the study of vaccinology where polyreactive antibodies could have some protective effect against related or non-related diseases, thus protecting the host with a single immunization against several pathogens, which has not only biological but also economic implications for vaccine production. In today's context, this is of great importance since SARS-CoV-2 colonizes the mucosas and global vaccination strategies, with few exceptions, are aimed at parenteral immunization.

Conclusion

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Polyreactive anti-polysaccharide IgA production using hybridoma has been reported for the first time. This polyreactivity was not only present against different serotypes of Neisseria meningitidis but also between Salmonella Typhi and N. meningitidis polysaccharides, which provides new data and a broad field of research on its implications for mucosal vaccines.

Conflict of Interest Statement
The authors declare no commercial or financial associations that could be construed as a potential conflict of interest.

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