Biotecnología Aplicada

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REPORT

B cells and CD8+ T cells interaction during the establishment of an anti-idiotypic response against the syngeneic monoclonal antibody P3

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ABSTRACT

P3 monoclonal antibody (P3 mAb) recognizes gangliosides carrying *N*-glycolylated sialic acid, sulphated glycolipids and antigens expressed in human breast, lung and melanoma tumors. This mAb generates a strong anti-idiotypic IgG isotype response in the BALB / c syngeneic model, even when administered in the absence of adjuvants or carrier proteins. However, the role of different populations of B cells in the anti-idiotypic response, the ability of P3 mAb to activate CD8+ T cells despite being a self-molecule, or if it carried a regulatory idiopeptide with effect on activated CD8+ T cells were unknown. In this work, it was demonstrated that P3 mAb is capable of recognizing and activating populations of B-1a and B-2 lymphocytes, with the participation of CD8+ T cells, which in turn were able to activate cytotoxic CD8+ T cells *in vitro*. The immunization with the mAb P3 recovered *in vivo* the frequency of CD8+ T lymphocyte populations in mice subjected to different immunosuppressive regimens, and generated a specific CTL response by an idiopeptide exclusive of this antibody. The results suggested the possible existence of an alternative mechanism to induce the regulation of the immune response against self-antigens through idiotypic interactions under physiological conditions, which involves B and CD8+ T cells. This demonstrates the ability of P3 mAb to activate cytotoxic CD8+ T lymphocytes, with potential for therapeutic treatments in immunosuppressed patients. This work received the Annual Award of the Cuban Academy of Sciences for the year 2017.

Keywords: anti-idiotypic antibody, CD8+ T cells, P3 mAb, regulatory idiopeptide, anti-idiotypic immunotherapy

RESUMEN

Interacción entre las células B y las células T CD8+ en el establecimiento de una respuesta anti-idiotípica contra el anticuerpo monoclonal singénico P3. El anticuerpo mocnoclonal P3 (AcM P3) reconoce a gangliósidos portadores de ácido siálico *N*-glicolilado, a glicolípidos sulfatados y a antígenos expresados en tumores humanos de mama, pulmón y melanoma. Este AcM genera una fuerte respuesta anti-idiotípica de isotipo IgG, en el modelo singénico BALB/c, incluso al ser administrado en ausencia de adyuvantes o proteínas transportadoras. Sin embargo, se desconocía el rol de las diferentes poblaciones de células B en la respuesta anti-idiotípica, la capacidad del AcM P3 de activar linfocitos T CD8+ a pesar de ser una molécula propia, o si este portaba un idiopéptido regulador con efecto sobre las células T CD8+ activadas. En este trabajo se demostró que el AcM P3 es capaz de reconocer y activar a las poblaciones de linfocitos B-1a y B-2, con la participación de células T CD8+, que a su vez fueron capaces de activar *in vitro* a células T CD8+ citotóxicas. La inmunización con AcM P3 recuperó *in vivo* la frecuencia de la población de linfocitos T CD8+, en ratones sometidos a diferentes regímenes inmunosupresores, y generó una respuesta CTL específica por un idiopéptido exclusivo de este anticuerpo. Los resultados sugirieron la posible existencia de un mecanismo alternativo para inducir la regulación de la respuesta inmune contra antígenos propios por interacciones idiotípicas en condiciones fisiológicas, que involucra a células B y T CD8+. Esto demuestra las capacidades del AcM P3 de activar linfocitos T CD8+ citotóxicos, con potencialidades para tratamientos terapéuticos en pacientes inmunosuprimidos. Este trabajo mereció el Premio Anual de la Academia de Ciencias de Cuba en el año 2017.

Palabras clave: anticuerpo anti-idiotípico, células T CD8+, AcM P3, idiopéptido regulador, inmunoterapia anti-idiotípica

How to cite (Vancouver style):
Martínez-Bedoya D, Hernández-Vázquez AM, Rondón-Corrales T, Griñán-Ramirez T, Rodríguez-Zhurbenko N, Pupo-Meriño A, *et al*. Interaction between B cells and CD8 + T cells in the establishment of an anti-idiotypic response against the syngeneic monoclonal antibody P3. Biotecnol Apl. 2018;35(2):2511-3.

Introduction

The P3 monoclonal antibody (P3 mAb) belongs to the gene family VHQ52, previously observed in autoantibodies against gangliosides and frequently used by B-1 lymphocytes. It recognizes gangliosides carrying *N*-glycolylated sialic acid, as well as sulphated glycolipids, but not to neutral glycolipids, nor the acetylated forms of gangliosides [1, 2]. It was also reported that this mAb recognizes antigens expressed in human breast, lung and melanoma tumors [1, 3, 4]. P3 mAb has the property of generating a strong anti-idiotypic IgG isotype response, in the syngeneic BALB/c mice strain, even when administered in the absence of adjuvants or carrier proteins [1]. The specific role of different populations of B cells in the anti-idiotypic response was not fully established.

1. Vazquez AM, Alfonso M, Lanne B, Karlsson KA, Carr A, Barroso O, *et al*. Generation of a murine monoclonal antibody specific for N-glycolylneuraminic acid-containing gangliosides that also recognizes sulfated glycolipids. Hybridoma. 1995;14(6):551-6.

It has been previously suggested[5] that the P3 mAb met the three criteria established by Paul and Bona [6, 7] to define a regulatory idiotope: to be immunogenic in the syngeneic model, to appear in antibodies with different specificities and to induce the activation of autologous T cells. Considering this, the ability mAb P3 to activate T cells, despite being a self-molecule, may be essential for its immunogenicity. Moreover, the participation of CD8+ T cells in the induction of the humoral response against P3 mAb has not been studied. In addition, it would be important to determine if indeed the P3 mAb carries a regulatory idiopeptide capable of activating CD8+ T cells with the ability to regulate the response against this antibody.

Therefore, this work was aimed at evaluating the participation of B-1a, B-2 and T CD8+ lymphocytes in the generation of an anti-idiotypic IgG isotype response against P3 mAb in the BALB/c model. Also, to determine the ability of P3 mAb to recognize and activate *in vitro* and *in vivo* peritoneal B-1a and splenic B-2 cells populations. An finally, to assess the ability of P3 mAb to activate CD8+ T cells *in vitro* and *in vivo* and identify whether P3 mAb possesses an idiopeptide capable of activating a CTL response. The results indicated the potential use of P3 mAb for immunotherapeutic approaches. Due to its relevance, this work received the Annual Award of the Cuban Academy of Sciences for the year 2017.

Results

Evaluation of the involvement of T lymphocytes in the generation of the anti-idiotypic response of isotype IgG against the P3 mAb in the BALB/c model

In contrast to the response observed in mice with intact T cell populations, no antibody response against the mAb was detected in the hyper-immune sera of the animals treated with anti-CD4a and anti-CD8a antibodies before the first dose of P3 mAb. These results suggested that the presence of CD8+ and CD4+ T cells is necessary at the time of induction of the response against the mAb. This was the first report implicating CD8+ T cells in the induction of an anti-idiotypic response *in vivo* under non-immunogenic conditions. This result opened up new possibilities for understanding the regulation and natural activation of idiotypic B-T cell networks [8].

Ability of P3 mAb to recognize and activate B cell populations

It was demonstrated that B-1a cells participate in the antibody response against P3 mAb, since unlike the results observed in BALB/c mice, no anti-idiotypic response was detected after four doses of P3 mAb in BALB/Xid mice. Moreover, P3 mAb was able to recognize on average 30 % of B-1a peritoneal cells. An increase in the percentage of B-1a cells expressing the activation markers CD25, CD69 and CD86 was observed, after being activated by immunization with P3 mAb. It was also proven that P3 mAb was able to activate *in vitro* naïve B-1a cells obtained from mice without any previous immunization. Incubation of naïve peritoneal B-1a cells with the P3 mAb for three days induced an increase in the percentage of cells expressing the activation markers CD25, CD69 and CD86. It also significantly increased the number of IgM spots per B-1a cells in an ELISPOT assay, indicating that a greater number of cells secrete IgM in response to stimulation with the antibody. The same effect was found in the percentage of cells B-1a as producing the cytokines tested: IFN-γ, IL-4 and IL-10. The combined transfer of B-1 and B-2 populations to the BALB/Xid mice was necessary to generate an idiotypic response against the P3 mAb [9]. As reported for B-1a cells, P3 mAb was able to specifically recognize B-2 spleen cells from naive mice, although at lower levels. Unlike for B-1a cells, no increase was observed in the percentage of B-2 cells expressing the CD25, CD69 and CD86 activation markers. Incubation with P3 mAb caused an increase in the activation markers CD25, CD69 and CD86 and produced a significant increase in the percentage of cells capable of producing IFN-γ and IL-4 but not IL-10. After incubation, activation markers CD25, CD69 and CD86 raised and the percentage of IFN-γ and IL-4-secreting cells significantly increased, but not for IL-4 [10].

Ability of P3 mAb to activate CD8+ T cells *in vitro* and *in vivo*

The incubation of CD8+ T cells with splenic B-2 cells from mice immunized with P3 mAb, without the addition of P3 mAb to the culture, did not generally induce a significant increase in activation markers. Nevertheless, when the P3 mAb was added to the culture, the *in vitro* re-stimulation did significantly increase the percentage of CD8+ T cells that expressed the activation markers CD25 and CD69, the CTL functional markers CD107a and granzyme B and that secrete IFN-γ. Surprisingly, the addition of the P3 mAb to the culture of CD8+ T cells with the B-2 cells of mice immunized with the control antibody also showed a stimulatory effect for CD107a and CD69 expression. These results suggested that P3 mAb has the ability to license naïve B-2 cells to elicit a certain level of activation of naïve CD8+ T cells, a property that is generally regarded as exclusive for dendritic cells. B-1a cells from mice immunized with P3 mAb also showed a significant increase in the percentage of CD8+ T cells expressing CD25 and CD107a or producing IFN-γ. In contrast to the results with B-2 cells, the addition of the P3 mAb to the culture had little effect on the activation of the CD8+ T cells, as compared with the addition of the control antibody [10].

In the case of B-1a cells obtained from mice immunized with the isotype control antibody, no activation of the CD8+ T cells was induced when the P3 mAb was added to the culture. These results suggested that the activation levels achieved by B-1a cells *in vivo*, together with the high percentage of B-1a cells recognized by P3 mAb, are sufficient to activate CD8+ T cells *in vitro* [10]. First, there was a significant increase in the percentage of CD8+ T lymphocytes in BALB/c mice suppressed and immunized with the P3 mAb, with respect to the control animals. Next, mAb P3 was able to induce a significant recovery of CD8+ T and CD4+ T cell subpopulations, but not of the B cell population in a lymphopenia model induced by cyclophosphamide [8].

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In the clinical setting, a fast reconstitution of T cell-dependent immunity, as observed with P3 mAb, is critical for patients with lympho-suppressive regimens. To evaluate the functionality of the T lymphocyte population generated after treatment with the P3 mAb, an *in vivo* model was used in which mice were immunosuppressed by the establishment of a syngeneic tumor. In BALB/c mice immunosuppressed by the syngeneic F3II tumor and treated with the P3 mAb, a significant decrease in the grafting frequency of the allogenic B16-F10 tumor was observed with respect to that shown by the unimmunized control group [8]. We identified, using bioinformatic tools, a peptide with the sequence MYYCARSGV in the VH region of P3 mAb, which binds specifically to the MHC class I alleles of the BALB/c strain of mice. The identified idiopeptide is unique to P3 mAb, since no other antibody reported in the Abysis database possesses that exact sequence. This peptide is capable of binding to at least eight HLA class I alleles, which are present in more than a third of the population of Cuba or the US. Immunization with P3 mAb was able to generate a CTL-specific response capable of significantly lysing only transferred spleen cells loaded with the peptide specific to the VH region of P3 mAb [10].

Relevance of the study

The results shown in this work allowed to postulate a model in the light of a physiological mechanism of regulation of the immune response against self glycolipid antigens, but also to provide the molecular and cellular bases to confirm the existence in the P3 mAb of a regulatory idiotope. Pérez *et al*. [5] suggested that the P3 mAb met the three criteria established by Paul and Bona to define a regulatory idiotope [6, 7]. Our work further provides an additional element or fourth criterion as to define a regulatory idiotope, consisting on the existence of specific cytotoxic CD8+ T cell population able to be activated by an idiotope peptide which plays a regulatory role on the anti-idiotypic response. On the other hand, the existence of peptides with the ability to bind MHC I molecules and generate a CTL response *in vivo* allows an operationalization of the regulatory idiotope concept, which could facilitate its identification in other antibodies and its potential use for modulating different types of immune responses.

In addition to the theoretical contribution, the present work has potential practical importance and social impact. Firstly, it contributes to the understanding of the action mechanisms of idiotypic vaccines such as the Vaxira vaccine developed at CIM, currently registered for the use in patients with lung cancer. On the other hand, it would allow a better design of new vaccines or anti-idiotypic antibodies as to modulate their immunogenicity and their ability to interact with immune cells. The knowledge derived from this work would be translated into the design of more effective treatments not only for cancer, but also for other diseases of high incidence, such as autoimmune and atherosclerosis diseases.