Importancia de los linfocitos T γδ en la respuesta inmunitaria de los bovinos

Importance of γδ T lymphocytes in the bovine immune response

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Abstract

The bovine γδ T lymphocytes conform a very important cell subset, not completely understood, which provides protective immune responses to the bovines. Their roles in non-specific and acquired immune responses of bovines are analyzed and discussed, including those of γδ T cells from other species.

Key words: BOVINE LYMPHOCYTES, IMMUNE RESPONSE, γδ T LYMPHOCYTES.

Resumen

Los linfocitos T γδ de los bovinos constituyen una subpoblación de células T importante, no completamente comprendida, que lleva a cabo respuestas inmunitarias protectoras de dichos rumiantes. Se analiza y discute su papel, tanto en la respuesta inmunitaria no-específica como en la adquirida de los bovinos, incluyendo la de células T γδ de otras especies.

Palabras clave: LINFOCITOS DE BOVINO, RESPUESTA INMUNITARIA, LINFOCITOS T γδ.

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Introduction

T lymphocytes are originated from lymphoid progenitors in the bone marrow hematopoietic tissue and differentiate in the thymus (primary lymphoid organ). Later, mature T cells go to the peripheral secondary lymphoid organs, including other lymphoid tissues (i.e. lymphatic ganglia, spleen and mucosa associated lymphoid tissue –MALT- among others) which virtually cover all the body, and to the circulation to conform a part of the peripheral blood lymphocyte pool. Bovines, as other vertebrate species, show two main T lymphocyte subpopulations: those which express the T cell receptor (TCR) $\alpha\beta$ for foreign antigens and those which express the TCR γδ. However, bovines have a proportion of peripheral γδ T lymphocytes greater than that observed in other vertebrate species.[1] In this context and based on observation, it has been suggested that T γδ lymphocyte percentages vary so much among the different vertebrate species that these can be classified as "γδ high" or "γδ low".[2] Humans and mice (2-5%) are considered "γδ low" [3,4] and chickens (15%)[5], swine (24%)[6] and cattle (20-40% up to 70% in newborns)[7-9] are included among "γδ high" (Figure 1).

The high concentration of γδ T cells in ruminants and swine is attributed to the presence of a γδ T cell subpopulation which express the molecule workshop cluster 1 (WC1) in ruminants and the orthologue in swine. WC1 and orthologues have been identified only in artiodactyls including ruminants, swine and camelids.[10,11] Available information suggests that this is a unique γδ T cell population which has evolved in artiodactyls. The concentration of lymphocytes T WC1- γδ identified in ruminants and swine is similar to that observed in humans and mice.[12,13] The analysis of the γδ T cells in chicken has not showed an explanation of the high concentration of γδ T cells. In this context, it has been demonstrated that the avian γδ T cells are heterogeneous, both in the characteristics of the CD8 antigen, as in the tissue localization and their functional characteristics such as the proliferation and expression of mRNA.[14] Recently cross-reaction antibodies for the study of subpopulations of lymphocytes γδ T in horses and many species of primates were characterized.[15] Other species in which γδ T lymphocytes have been identified include sheep,[16] goats,[17] dogs,[18] cats,[19] rats,[20] rabbits,[21] and Guinea pigs.[22]

γδ T lymphocytes are the first T cells which develop; they are found at body entrance sites (tissues associated with epithelial cells, such as intestine, lung mucosa and skin), they accumulate during inflammation and are involved in immune responses against a wide array of pathogenic agents. It has been indicated that in all vertebrate species studied, γδ T lymphocytes are

Introducción

Los linfocitos T se originan de los progenitores linfoides en el tejido hematopoyético de la médula ósea y se diferencian en el timo (órgano linfoide primario). Las células T maduras después se dirigen a los órganos linfoides secundarios periféricos, incluyendo otro tejido linfoide (por ejemplo, los ganglios linfáticos, bazo y tejido linfoide asociado con mucosas –MALT, entre otros) que virtualmente cubren todo el cuerpo, y también a la circulación para conformar parte de los linfocitos recirculantes. Los bovinos, como otras especies de vertebrados, presentan dos subpoblaciones principales de linfocitos T: los que expresan el receptor de T (TCR) $\alpha\beta$ para antígenos extraños y los que expresan el TCR γδ. Sin embargo, los bovinos tienen una proporción de linfocitos T γδ circulantes mucho más grande que la que se observa en otras especies.[1] En este sentido, se ha sugerido, con base en la observación, que los porcentajes de linfocitos T γδ en sangre periférica varían tanto entre las distintas especies de vertebrados que éstas pueden ser clasificadas como "γδ alta" o "γδ baja".[2] Entre las especies "γδ baja" se incluye a los humanos y ratones (2-5%)[3, 4] y en las especies "γδ alta" se incluye a las gallinas (15%),[5] cerdos (24%)[6] y ganado bovino (20-40% y hasta 70% en neonatos)[7-9] (Figura 1).

La concentración alta de células T γδ en rumiantes y cerdos se atribuye a la presencia de una subpoblación de células T γδ que expresa la molécula *workshop cluster 1* (WC1) en rumiantes y el ortólogo en cerdos. WC1 y ortólogos han sido identificados solamente en artiodáctilos, incluyendo rumiantes, cerdos y camélidos.[10, 11] La información disponible actualmente sugiere que ésta es una población única de células T γδ que ha evolucionado en artiodáctilos. La concentración de linfocitos T WC1- γδ identificada en rumiantes y cerdos es similar a la concentración observada en humanos y ratones.[12, 13] El análisis de las células T γδ en gallinas no ha revelado una explicación de la alta concentración de las células T γδ. En este contexto, se ha demostrado que las células T γδ de ave son heterogéneas, tanto en las características del antígeno CD8, como en la localización tisular y sus características funcionales como la proliferación y la expresión de ARNm.[14] Recientemente se caracterizaron anticuerpos de reacción cruzada para el estudio de las poblaciones de linfocitos T γδ en caballos y muchas especies de primates.[15] Otras especies en las que se ha identificado linfocitos T γδ son borregos,[16] cabras,[17] perros,[18] gatos,[19] ratas,[20] conejos[21] y cuyes.[22]

Los linfocitos T γδ son los primeros linfocitos T que se desarrollan; se pueden encontrar en sitios de entrada al organismo (tejidos asociados con células epiteliales, tales como el intestino, la mucosa pulmonar

abundantly present in epithelia and that great part of their functions are unknown.[23]

Are γδT lymphocytes the link between the innate immune response and the acquired immune response?

It has been pointed out that many of the γδ T lymphocytes appear to be directed against pathogenic agents such as bacteria, viruses and parasites[24] and inclusive it has been suggested that γδ T lymphocytes might represent the first step of acquired immunity in evolution, reinforcing the gastrointestinal defense against microbial invasion as a result of an increased traumatism by lesions and infections when the first host fishes developed a mandible.[25] In this context, it has been observed that γδ T lymphocytes from bovines directly respond to the pathogen-associated molecular patterns (PAMP) through the expression of receptors for PAMP, suggesting that γδ T lymphocytes play a relevant role in the innate immune response[26] and it has been proposed that they function as a link between the innate and acquired immune responses.[27-29] In the tissues associated with epithelial cells and inflammation sites, the immune system's innate cells, such as myeloid cells, epithelial cells, dendritic cells and some specialized T cells, including γδ T lymphocytes, may detect invasive microbes through the recognizing of PAMP. In γδ T cells, PAMPs such as a lipopolysaccharide crude preparations (LPS), induce the selective expression of some chemokines such as the macrophage inflammatory protein-1a (MIP-1γδ) and the MIP-1γδ.[26] In the global analysis of gene expression, it has been observed that bovine γδ T lymphocytes express transcripts for the different

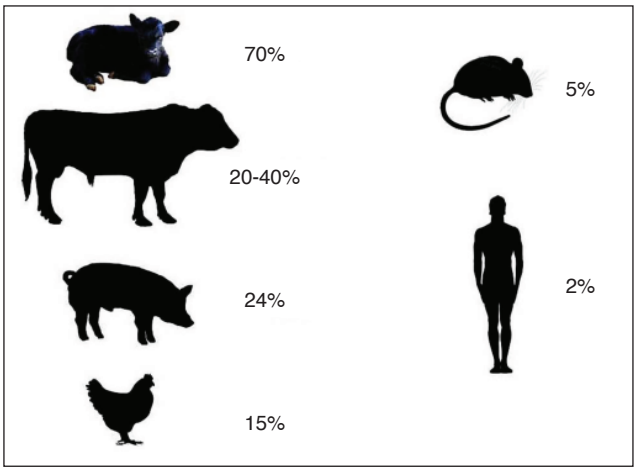

Figura 1. Porcentajes de linfocitos γδ T descritos en bovinos: (7, 8, 9), cerdos (6), gallinas (5), humanos (3) y ratones (4).

Figure 1. Percentages of γδ T lymphocytes described in bovines: (7, 8, 9), swine (6), chickens (5), humans (3) and mice (4).

y la piel), se acumulan durante la inflamación y están involucrados en las respuestas inmunitarias contra un amplio espectro de agentes patógenos. Se ha indicado que en todas las especies de vertebrados estudiadas hasta la fecha, los linfocitos T γδ están presentes de manera abundante en los epitelios y que la mayoría de sus funciones se desconoce.[23]

¿Son los linfocitos T *γδ* el eslabón entre las respuestas inmunitarias innata y adquirida?

Se ha señalado que muchas de las respuestas de los linfocitos T γδ parecen estar dirigidas contra agentes patógenos como bacterias, virus y parásitos[24] e incluso se ha sugerido que los linfocitos T γδ pudieran representar el primer paso en la evolución de la inmunidad adaptativa, reforzando la defensa gastrointestinal contra la invasión microbiana como resultado de un traumatismo incrementado por las lesiones e infecciones cuando los primeros peces hospederos desarrollaron una mandíbula.[25] En este sentido, se ha observado que los linfocitos T γδ de bovino responden directamente a los patrones moleculares asociados con patógenos (PMAP) a través de la expresión de receptores para PMAP, por lo que se ha sugerido que los linfocitos T γδ desempeñan un papel relevante en la respuesta inmunitaria innata[26] y se ha propuesto que actúan como eslabón entre las respuestas inmunitarias innata y adquirida.[27-29] En los tejidos asociados con células epiteliales y sitios de inflamación, las células del sistema inmunitario innato, tales como las células mieloides, células epiteliales, células dendríticas y algunas células T especializadas, incluyendo las células T γδ, pueden encontrar microbios invasores vía reconocimiento de PMAP. En las células T γδ, los PMAP, tales como preparaciones con lipopolisacárido crudo (LPS), inducen la expresión selectiva de algunas quimiocinas, como la proteína inflamatoria de macrófago-1γδ (MIP-1γδ) y la MIP-1γδ.[26] En el análisis global de expresión de genes, se ha observado que los linfocitos T γδ de bovino expresan transcritos para distintos receptores de PAMP, incluyendo receptores carroñeros (scavenger), como el CD36, receptores tipo Toll y CD11b, entre otros.[29] Aunque recientemente se demostró la expresión de CD36 en linfocitos T γδ de bovino, se ha señalado, sin embargo, que la importancia de estos receptores en las respuestas a PMAP por dichas células, no ha sido suficientemente explorada.[29] Se ha sugerido que la respuesta a PMAP induce una sensibilización de células T γδ que da por resultado una respuesta más vigorosa a vías de señalización de citocinas y antígeno. Los linfocitos T γδ activados por PMAP se definen por la regulación de un número selecto de citocinas, que incluyen MIP 1 alfa y MIP 1

PAMPs receptors, including scavenger receptors, such as CD36, Toll-like receptors and CD11b, among others.[29] Although recently it was demonstrated the expression of CD36 in bovine γδ T lymphocytes, it has been pointed out however, that the importance of these receptors in the responses of such cells to PAMPs has not been sufficiently explored[29] It has been suggested that the response to PAMPs induces priming of γδ T cells which gives rise to a strong response to signaling cytokines´ pathways and to antigen. γδ T lymphocytes activated by PAMPs are defined by the regulation of a select number of cytokines, which include MIP 1 alpha and MIP 1 beta, and for antigens such as the surface IL-2 receptor alpha (IL-2Ralpha) and CD69, in the absence of a prototypic marker of an activated γδ T cell, the IFN-γδ. The γδ T cells activated by PAMPs are more capable to proliferate in response to IL-2 or IL-15 in the absence of antigen. Similarly, the PAMPs such as endotoxin, peptidoglycan and beta-glucan are effective agents for priming γδ T cells; however, the more potent antigen-independent agonists defined to date, are the condensed oligomeric tannins produced by some plants.[30] In this context, it has been demonstrated that the genome of bovines (Btau_3.1) contains a wide and diverse array of genes for the T cell delta receptor (TRD) when it is compared with the genomes of "γδ T low" species, which suggests that the bovine γδ T cells play an important role in the immune response, since it can be predicted that such cells bind a great variety of antigens.[31]

Role of bovine *γδ* T lymphocytes in infections caused by pathogenic agents

With respect to the previous information, it has been observed that γδ T cells take action against diverse pathogenic agents: viruses, bacteria and parasites. Table 1 shows some of the pathogens recognized by bovine γδ T lymphocytes.

In this context, it is important to point out the interest in the study of mycobacterial infections in bovines.[27, 36, 37]

γδ T lymphocytes' functions

Interest for knowing the functions of γδ T lymphocytes, particularly those of ruminants, has boosted the research in this area during the last two decades, which has resulted a better understanding of the biological activities of this kind of lymphocytes, including antigen presentation to other lymphocytes, induction of effector cells, immune memory, modulation of the immune response, cytokines' production, recognizing of conserved molecules on pathogens and mucosal immune surveillance (Table 2).

beta, y por antígenos, como el receptor de superficie alfa de IL2 (IL-2R alpha) y CD69, en ausencia de un marcador prototípico de una célula T γδ activada, el IFN-γδ. Las células T γδ activadas por PMAP son más capaces de proliferar en respuesta a IL-2 o IL-15 en ausencia de antígeno. Asimismo, los PMAP como la endotoxina, el peptidoglicano y el beta-glucano son agentes efectivos para sensibilizar células T γδ, pero los más potentes agonistas antígeno-independiente, definidos hasta la fecha, son los taninos oligoméricos condensados producidos por algunas plantas.[30] En este contexto, se ha demostrado que el genoma de los bovinos (Btau_3.1) contiene un repertorio grande y diverso de genes del receptor delta de las células T (TRD), cuando se compara con los genomas de las especies "T γδ baja", lo que sugiere que las células T γδ de bovino tienen un papel importante en la función inmunitaria, puesto que se podría predecir que dichas células se unen a una gran variedad de antígenos.[31]

Papel de los linfocitos T *γδ* de bovino en infecciones por agentes patógenos

En relación con el apartado anterior, se ha observado que los linfocitos T γδ actúan contra diversos agentes patógenos: virus, bacterias y parásitos. En el Cuadro 1 se presentan algunos de los patógenos que son reconocidos por los linfocitos T γδ de bovino.

Con respecto al estudio de agentes patógenos en bovinos y linfocitos T γδ, llama la atención el interés mostrado a las infecciones por micobacterias.[27,36,37]

Funciones de los linfocitos T *γδ*

El interés por conocer las funciones de los linfocitos T γδ, particularmente de rumiantes, ha estimulado la investigación en esta área en las últimas dos décadas, lo que ha dado por resultado un mayor conocimiento de las actividades biológicas de estos linfocitos, entre las que se incluyen la presentación de antígeno a otros linfocitos, inducción de células efectoras, memoria inmunitaria, modulación de la respuesta inmunitaria, producción de citocinas, reconocimiento de moléculas conservadas en patógenos y vigilancia inmunitaria en mucosas (Cuadro 2).

¿Es posible inducir respuestas inmunitarias protectoras a través de la activación de linfocitos T *γδ*?

Se ha indicado que en todas las especies estudiadas hasta la fecha, los linfocitos T γδ están presentes de manera abundante en epitelios, como son los de los tractos respiratorio,[55] gastrointestinal,[56,57] reproduc-

Is it possible to induce protective immune responses through the activation of *γδ* T lymphocytes?

In all species studied to date it has been indicated that the γδ lymphocytes are abundantly present in epithelia such as those of respiratory,[55] gastrointestinal[56,57] and reproductive[58,59] tracts and in the skin; [9,23,60,61] similarly, it has been pointed out that many of this subpopulation of T lymphocytes' functions are still unknown.[62-64]

Handling of the immune response throughout the activation of *γδ* T lymphocytes

The interest for knowing how the different cells of the immune system act and how these may be artificially stimulated, is the basis for the development of vaccines or immunotherapies for preventing or controlling the diverse diseases which affect man and his domestic animals. In this context, in different assays it has

tivo[58,59] y piel;[9,23,60,61] asimismo, se ha señalado que muchas de las funciones de esta subpoblación de linfocitos T son todavía desconocidas.[62-64]

Manipulación de la respuesta inmunitaria a través de la activación de linfocitos T *γδ*

El interés por conocer de qué manera actúan las diferentes células que conforman el sistema inmunitario y de qué forma pueden ser estimuladas artificialmente, es la base para desarrollar vacunas o inmunoterapias para prevenir o controlar las distintas enfermedades que afectan al hombre y sus animales domésticos. En este contexto, se ha demostrado en distintos ensayos que la vacunación contra diferentes agentes patógenos es capaz de inducir linfocitos T γδ protectores. En el caso de los bovinos, se ha demostrado que los linfocitos T γδ de animales vacunados, pero no los de bovinos no-vacunados con un herpesvirus 1 (BHV1) vivo modificado, mostraron un aumento

Cuadro 1

Papel de los linfocitos T γδ en enfermedades producidas por diversos agentes patógenos

Role of γδ T lymphocytes in diseases produced by diverse pathogenic agents

Species	*Pathogenic agent*	*Relevant characteristics*	*References*
Bovine	Bovine viral diarrhea virus (BVD)	Induction of γδ T in calves by maternal antibodies	32
Bovine	Bovine Papillomavirus type 4 (BPV-4)	Implicated in papilloma regression	33
Bovine	*Anaplasma marginale*	Recognizing of MSP2	34
Bovine	*Cowdria ruminantium*	Probable role in protective immune response	35
Bovine	*Mycobacterium bovis*	Induction of interferon-γ production with *Mycobacterium* products	36
Bovine	*Mycobacterium avium* subsp. *paratuberculosis*	Probable role in the regulation of Th1 type responses	37
Bovine	*Staphylococcus* and *Streptococcus* mastitis	Involved in the protective immune response at the mammary gland	38
Bovine	*Babesia* spp	Implicated in age resistance	39
Bovine	*Theileria annulata*	Involved with the protective immune response	40
Bovine	*Dyctiocaulus viviparus*	Implicated in the protective immune response at the lung	41
Bovine	*Fasciola hepatica*	Regulate T *αβ* lymphocytes which may have a non-protective role	42

Cuadro 2

Funciones estudiadas en linfocitos T γδ de diferentes especies animales

Studied functions in γδ T lymphocytes from different animal species

Species	*Studied function*	*Reference*
Bovine	Antigen (Ag) presentation to T $\alpha\beta$	43
Human	Ag presentation γδ T lymphocytes	44
Human	Ag presentation and induction of effector T $\alpha\beta$ cells	45
Human	Immunememory to *Mycobacterium*	46
Bovine	Regulation of the immune response	47
Bovine	Modulation of the immune response to pathogens	48
Bovine	Induction of Th1 cytokines' production in calves	49
Mouse	IL-17 production	50
Bovine	Interferon gamma production	36, 51
Bovine	The treatment of calves with dexamethasone reduces peripheral T γδ lymphocyte numbers	52
Bovine	The GD3.5-(CD8+) γδ T subpopulation functions as mucosal sentinel	53
Bovine	Recognizing of pathogen associated molecular patterns	30
Bovine	Cytotoxicity mediated by T WC1+γδ lymphocytes	54

been demonstrated that vaccination against various pathogenic agents is capable of inducing protective γδ T lymphocytes. In bovines, it has been demonstrated that γδ T lymphocytes from vaccinated animals with a live modified hespervirus 1 (BHV1), but not from the non-vaccinated bovines, showed a significant increase in the expression of CD25 when such cells were incubated *in vitro* with BHV1.[65] Similarly, it has been observed that immunization of bovines with *Cowdria ruminantium* induces γδ T lymphocytes with protective activity.[35] In a series of experiments in bovines it was demonstrated that protective T WC1+ γδ lymphocytes are generated in response to the vaccination against *Leptospira borgpetersenii* [66-68]and that the protective response is directed to the uterus, target organ of the *Leptospira* infection, coinciding with the observation that γδ T lymphocytes represent the highest population of T lymphocytes in the ruminant uterus.[58,59] Besides, the vaccination of bovines with the inactivated *Leptospira* vaccine induces a memory population of T WC1+ γδ lymphocytes.[68] In addition, it has been suggested that in humans vaccinated with BCG (*Mycobacterium bovis* - Bacillus Calmette-Guérin), memory γδ T lymphocytes are developed which cross react with antigens present in the microorganisms of significativo en la expresión de CD25 cuando se incubaron *in vitro* con BHV1.[65] Similarmente, se ha observado que la inmunización de bovinos con *Cowdria ruminantium* induce linfocitos T γδ con actividad protectora.[35] En otra serie de experimentos se demostró que los linfocitos T WC1+ γδ protectores son generados en respuesta a la vacunación contra *Leptospira borgpetersenii* en bovinos[66-68] y que la respuesta protectora está dirigida al útero, órgano blanco de la infección por *Leptospira*, lo que coincide con la observación de que los linfocitos T γδ representan la mayor población de linfocitos T en el útero de los rumiantes.[58,59] Además, la inmunización de bovinos con la vacuna muerta de *Leptospira* induce una población de linfocitos T WC1+ γδ de memoria.[68] Asimismo, se ha sugerido que en humanos vacunados con BCG (Bacilo Calmette-Guérin) de *Mycobacterium bovis* se desarrollan células T γδ de memoria, que dan reacción cruzada con antígenos presentes en los microorganismos del complejo *Mycobacterium tuberculosis*.[46] En este orden, otros investigadores demostraron que los linfocitos T γδ de cerdos jóvenes son amplificados funcionalmente por la inmunización con la vacuna BCG de *M. bovis*, sugiriendo que dicha subpoblación celular desempeña un papel importante en la res-

the *Mycobacterium tuberculosis* complex.[46] In this order, other researchers demonstrated that γδ T lymphocytes from young pigs are functionally amplified by the vaccination with the *M. bovis* BCG vaccine, suggesting that such cell subpopulation carries out an important role in the acquired immune response generated by the vaccination with BCG. [69] In the same way, it has been demonstrated that human primed and amplified γδ T cells, through dendritic cells infected with BCG, act as a memory cytotoxic cell population that express a high amount of perforin and which is efficient killing monocytes infected with *Mycobacterium.* [70]

In other assay it was demonstrated that the vaccination of humans with the live vaccine against the canary smallpox virus, induces γδ T lymphocytes that produce interferon-γδ in an increased manner, suggesting that a memory type 1 immune response might be amplified. [71] In this context, studies carried out in mice suggest that γδ T lymphocytes conform a population induced by the immunization to *Plasmodium yoelii* irradiated sporozoites, which is capable to diminish the pre-erythrocytic load, which would represent a significant effector cell population that may be induced through the vaccination. [72]

On the other hand, it has been observed that Th1 immunity induced by BCG-infected dendritic cells on virgin T CD4 cells was increased by γδ T lymphocytes activated with drugs such as BrHpp (bromohydrin-pyrophosphate) or Zol (zoledronate), suggesting that drugs which activate γδ T cells might be used to amplify Th1 type immunity induced by BCG.[73] γδ T lymphocytes share similar functions with dendritic cells, such as antigen capture and antigen presentation,[74] and with other innate lymphocytes, for example NK and NK-T cells, the cytotoxic and tumoricidal activity in addition to the stimulation and maturing of dendritic cells.[75,76] In this sense, it has been suggested the handling of the immune system through the stimulation of γδ T lymphocytes to amplify maturing of dendritic cells by using non-peptidic molecules derived from different microorganisms with the idea of developing new vaccines or immunotherapies.[77,78]

On the basis of the previous information, it would be appropriate to find out if the protective immunity conferred by the inoculation of *Lactobacillus casei* into mice against the parasites *Trichinella spiralis*, in which an increase in interferon-γδ (IFN-γδ) production was observed,[79] and *Babesia microti,*[80] it depends, and in what degree, on the stimulation of γδ T lymphocytes. Similarly, the observation that the inoculation of *L. casei* two days before the application of a vaccine against bovine babesiosis, boosts the efficiency of this vaccine at challenge[81] suggests to evaluate the role of such a cellular population in the protection, considering the increase in the production of IFN-γδ (as determined

puesta inmunitaria adquirida, generada por la vacunación con BCG.[69] Asimismo, se ha demostrado que los linfocitos T γδ de humano sensibilizados y amplificados, vía células dendríticas infectadas con BCG, se manifiestan como una población de células citotóxicas de memoria que expresan una cantidad elevada de perforina, y que son eficientes matando monocitos infectados con micobacterias.[70]

En otro ensayo se demostró que la vacunación con la vacuna viva contra el virus de la viruela del canario en humanos, induce linfocitos T γδ que producen Interferon-γδ de manera incrementada, lo que sugiere que se pudiera amplificar una respuesta inmunitaria de memoria tipo 1.[71] En este contexto, estudios llevados a cabo en ratones sugieren que linfocitos T γδ protectores constituyen una población inducida por la inmunización con esporozoitos irradiados de *Plasmodium yoelii*, que es capaz de disminuir la carga parasitaria pre-eritrocítica, lo que representaría una población efectora significativa que puede ser inducida por la vacunación.[72]

Por otro lado, se ha observado que la inmunidad tipo Th1 inducida por células dendríticas infectadas con BCG sobre células T CD4 vírgenes, fue incrementada por células T γδ activadas con fármacos como BrHpp (bromohydrin-pyrophosphate) o Zol (zoledronate), lo que sugiere que las drogas que activan a células T γδ podrían ser utilizadas para amplificar la inmunidad tipo Th1 inducida por BCG.[73] Los linfocitos T γδ comparten funciones similares con las células dendríticas, como la captación y presentación de antígeno,[74] y con otros linfocitos innatos, NK (asesinos naturales) y NK-T (T asesinos naturales), la actividad citotóxica y tumoricida, además de la estimulación de la maduración de células dendríticas.[75,76] En este sentido, se ha sugerido la manipulación del sistema inmunitario a través de la estimulación de los linfocitos T γδ para amplificar la maduración de las células dendríticas por medio del uso de moléculas no peptídicas derivadas de diferentes microorganismos con el objeto de desarrollar nuevas vacunas o inmunoterapias.[77,78]

Con base en lo anterior, sería conveniente averiguar si la inmunidad protectora inespecífica conferida por la inoculación de *Lactobacillus casei* en ratones contra los parásitos *Trichinella spiralis*, en donde se observó un incremento en la producción de interferon-γδ[79] y *Babesia microt,*[80] depende, y en qué grado, de la estimulación de linfocitos T γδ. Asimismo, la observación de que al inocular *L. casei* dos días antes de la aplicación de la vacuna mixta contra babesiosis bovina, se incrementa la efectividad de la misma al desafío[81] sugiere evaluar la participación de dicha población celular en la protección ya que se registró un incremento de la producción de interferon-γδ (determinado por PCR en tiempo real) en los grupos de animales tratados solamente con *L. casei* y con *L.*

by real time PCR) observed in the groups of bovines treated only with *L. casei* or with *L. casei* and the vaccine, as compared with the control and only vaccine treated groups. In this context, it has been indicated that the γδ T lymphocytes are IFN-γδ producers. [36, 68]

The analyzed information shows that γδ T lymphocytes make up a T cell population, which carries out diverse activities such as immune response regulation, cytokines production, cytotoxic activity, antigen presentation, recognizing of pathogen-associated molecular patterns, immune memory, among others and that these cells interact with other immune cells such as dendritic cells and NK and NK-T lymphocytes. In the case of the bovine Btau3.1 genome, it has been demonstrated the existence of 13 members in the WCI gene family and it is probable that its diversity takes part in the functional differences observed among γδ T lymphocytes' populations.[31] However, there are many functions to be known and to define what are their roles in the immune response with the objective of designing new vaccines and immunotherapies for controlling bovine diseases. As for example, one of the suggested activities for bovine γδ T lymphocytes is their involvement in the age-protection against *Babesia* spp infection,[39] which has not been experimentally demonstrated; fact that would have important implications for the control of bovine babesiosis.

Related of the capacity of γδ T lymphocytes to generate a wide variety of antigen receptors[82, 83] it has been proposed that these cells carry out an important role in various homeostatic of innate and acquired-immunity[84] and non-immune processes, as well in pathologic situations through the recognizing of different antigens, infections' control and modulation of the tumor development.[85] In this context, recently it was demonstrated that human γδ T lymphocytes are capable of inducing strong responses involving CD8+ γδ T lymphocytes.[86] In the same way, it has been demonstrated that γδ T lymphocytes provide an IFN-γδ early essential stimulus to condition dendritic cells for an efficient priming of T CD8+ lymphocytes and the full development of a protective response,[87] fact which not only elucidate, in part, the roles of γδ T lymphocytes and dendritic cells in the interactions between the early immune response and the posterior acquired immune response, but also it helps for the innovative designing for the development of an efficient vaccine against tuberculosis –administrated by mucosal via- through the handling of γδ T lymphocytes.[88]

With respect to the previous information, in a recent study it was proved that human eosinophils express a γδTCR/CD3 receptor with similar characteristics to the γδTCR of γδ T lymphocytes. The authors of the research have proposed that such a receptor contributes to

casei y la vacuna, en comparación con los grupos de bovinos testigo y solo tratados con la vacuna. En este contexto, se ha indicado que los linfocitos T γδ son productores de interferon-γδ.[36,68]

La información analizada indica que los linfocitos T γδ conforman una población de células T que desempeña diversas actividades como son la regulación de la respuesta inmunitaria, producción de citocinas, actividad citotóxica, presentación de antígeno, reconocimiento de patrones moleculares asociados con patógenos, memoria inmunitaria, entre otras y que interaccionan con otras células inmunitarias como las células dendríticas y los linfocitos NK y NK-T. En el caso del genoma bovino Btau_3.1, se ha demostrado la existencia de 13 miembros en la familia de genes WCI y es probable que su diversidad contribuya a las diferencias funcionales que se han observado entre las poblaciones de células T γδ.[31] Sin embargo, todavía quedan por conocer muchas funciones y establecer con certeza cuál es su participación en la respuesta inmunitaria, con el objeto de diseñar nuevas vacunas e inmunoterapias para el control de enfermedades de los bovinos. Por ejemplo, una de las actividades que se han sugerido para los linfocitos T γδ de bovino, es su participación en la protección de edad contra la infección por *Babesia* spp,[39] pero que no se ha demostrado experimentalmente, lo que tendría implicaciones importantes para el control de la babesiosis bovina.

En cuanto a la capacidad de los linfocitos T γδ de generar una gran variedad de receptores de antígeno[82,83] se ha propuesto que estas células desempeñan un papel importante en una variedad de procesos homeostáticos de inmunidad innata y adaptativa[84] y no-inmunitarios, así como en situaciones patológicas a través del reconocimiento de diferentes antígenos, control de infecciones y modulación del desarrollo de tumores.[85] En este sentido, recientemente se demostró que los linfocitos T γδ de humano son capaces de inducir respuestas robustas de linfocitos efectores CD8+ Tγδ.[86] Similarmente se ha demostrado que los linfocitos T γδ proporcionan un estímulo temprano esencial de IFN-γ que condiciona a las células dendríticas para una sensibilización eficiente de linfocitos T CD8+ y el pleno desarrollo de una respuesta protectora,[87] lo que no solamente dilucida en parte el papel de los linfocitos T γδ y las células dendríticas en las interacciones entre las respuesta inmunitaria temprana y la respuesta inmunitaria adaptativa posterior, sino que también ayuda en el diseño de enfoques novedosos para el desarrollo de una vacuna eficiente contra tuberculosis –administrada vía mucosas– por medio de la manipulación de linfocitos T γδ.[88]

Con relación a lo anterior, en un estudio reciente se demostró que los eosinófilos de humano expresan

the innate responses to *Mycobacterium* and to tumors and may represent an additional interaction between myeloid cells and lymphoid cells;[89] this finding elucidates a little bit more the complex vertebrate immune response.

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un receptor γδTCR/CD3 con características similares al receptor γδTCR de los linfocitos T γδ. Los autores han propuesto que dicho receptor contribuye a las respuestas innatas contra micobacterias y tumores e incluso puede representar una interacción adicional entre células mieloides y células linfoides,[89] hallazgo que esclarece un poco mas el funcionamiento de la compleja respuesta inmunitaria de los vertebrados.

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