American Journal of Immunology 9 (2): 48-57, 2013
ISSN: 1553-619X

doi:10.3844/ajisp.2013.48.57 Published Online 9 (2) 2013 (http://www.thescipub.com/aji.toc)

Immunological Response in Bovine Lymph Nodes Stimulated with Subunits Vaccines

[1]Gabriel Andres Tafur Gomez, [1]Marlene Isabel Vargas, [1]Joaquin Hernan Patarroyo Salcedo, [1]Elisangela Neves de Souza, [1]Sidimar Sossai, [1]Leandro de Arujo, [2]Ana Paula Peconik and [1]Karlos Henrique Martins Kalks

[1]Department of Veterinary, Laboratory of Biology and Control of Haematozoa and Vectors, Federal University of Viçosa, Viçosa, MG, Brazil
[2]Department of Veterinary, Sector of Preventive Veterinary, Federal University of Lavras, Lavras, MG, Brazil

Received 2013-02-07, Revised 2013-06-20; Accepted 2013-06-20

ABSTRACT

The vaccination process belongs to the public health intervention methodologies that help prevent infections. Vaccinations performed successfully in the history of medicine reported the significance of this procedure to increase the quality of life, prevent zoonoses and improve animal production. Vaccine emergence remained without exact rules for a long time, maintaining a close relationship with pathogens. However, subunit vaccines, with a difference from the classical idea of protective immunity with microorganisms showed it is possible to trigger T-dependent responses with peptide, revealing new rules for vaccine development. This vaccination process starts by the modulation chance of adaptive immune response through peptide sequences process by APCs for immune synapse formation interceded for pMHC-TCR as a scaffold to T cells priming. In this way the immunological signal triggered by immune synapses is amplified in lymph nodes. As a consequence, T and B cells modulated by peptide activity interact between the B cell follicles region and T cell aggregates, which constitute the paracortical region of secondary lymphoid tissue to form connate unions as a prerequisite for clonal amplification and subsequent immunological memory. Indicating the knowledge of the mechanisms of immune response generated by peptides immunization is essential for understanding modulation, amplification and immune protection as demands for good subunits vaccine.

Keywords: Bovine Lymph Nodes, Synthetic Peptides, Recombinant Peptides, Immune Response

1. INTRODUCTION

The basic rules for vaccine development were proposed by Louis Pasteur after discovering that infections were caused by microbes, he argued that in order to make a vaccine, isolation, inactivation and inoculation of the disease microorganism, should be performed. These principles served to support for vaccines development, which became a powerful tool in less than a century, helping to eliminate some of the most devastating infectious diseases worldwide (Plotkin and Plotkin, 2011). However, with the new pathogens and vectors discovery, re-emergence of many microorganisms, coupled with increased resistance to infectious entities, as well as the pathogenicity reversion and low immune protection obtained by traditional vaccines; showed the research need to develop new products that would be safer and would induce effective protection against pathogens.

Indeed, the advances in immunology and biotechnology, led to greater understanding of immune response, together with the new public health paradigms, transferred the need to eliminate the biologics undesirable effects developed by conventional methodologies. That in order to improve vaccine

Corresponding Author: Joaquin Hernan Patarroyo Salcedo, Department of Veterinary, Laboratory of Biology and Control of Haematozoa and Vectors, Federal University of Viçosa, Viçosa, MG, Brazil Tel: +55 31 38992910; Fax: +55 31 38992864

development started the need to retain only protective pathogens immunogenic fraction, eliminating the fragments that were not necessary for protection, but were able to cause adverse reactions (Desmettre, 2011).

Therefore, employing methodologies which aided characterize the pathogens surface proteins, enabled peptides use to protective immunity against a particular pathogen, which thanks to advances in synthesis solid phase peptide automation was possible revoke laborious protein fractionation techniques which compromised the obtained material purity (Strugnell *et al*., 2011).

Adopting this new approach, researchs were conducted for developed synthetic peptides as immunogens for human vaccines. For this purpose, were inoculated synthetic antigens of Tobacco Mosaic Virus (TMV) in rabbits. Subsequently, could be observed viral neutralization and viral native proteins recognition by antibodies obtained from immunized animals (Neto *et al.*, 2013). This approach, allowed to goes beyond Pasteur's rules, serving as scaffold for developing novel peptides as potential immunogens for diseases control (Sette and Rappuoli, 2010). Nonetheless, only in 1981 this approach was considered for vaccine development in veterinary medicine, when it was careful as a support researh for the vaccine progress against feline rhinotracheitis (De Mestre, 2011).

Subsequently, computational prediction studies, based on primary structure and biochemical properties of proteins, began to be employed in antigenic and immunogenic protein sites identification as vaccine candidates. The bioinformatics techniques also contributed to epitope mapping studies of B and T cells, restricted to stimulation of Peripheral Blood Mononuclear Cells (PBMC) by proteins and synthetic peptides *in vitro*, to isolate the peptides with higher cell recognition capacity (Oliveira, 2006; Grimm *et al*., 2013). Encouraging the study of a new vaccines generation that offered greater safety and immunogenicity.

On the other hand, the methodologies based on genome research, allowed sequenced complete microorganism genome in 1995, enabled the genome access for protein expression. This new revolution aproach, aided the peptides design based on genome information. This methodology was called "reverse vaccinology" and actually served as support for recombinant vaccines assays (Seib *et al*., 2012).

In this context, the understanding of immune response mechanisms in lymph nodes, triggered after peptides immunization, provides important information about immunization efficient with different types of vaccines, including subunits vaccines. So in this review will present the immune evens founded in bovine lymph nodes immunized with immunogens peptides against tick *Rhipicephalus microplus* as model.

1.1. Subunit Vaccines Against Ticks

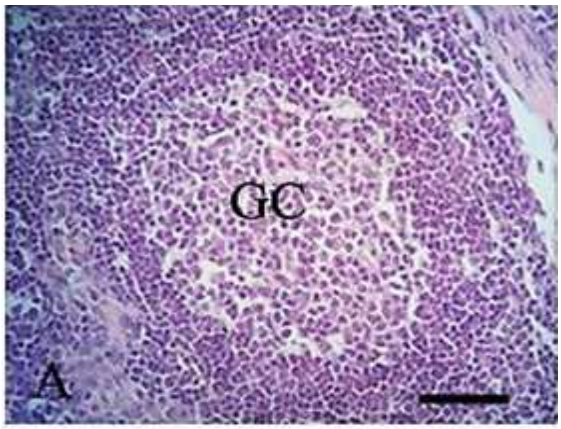
After intensified-experiments with subunit vaccines against microorganismes, were performed studies to discover new molecules capable to generating adverse effects against ticks. Indeed, the antigens obtained from midgut of *R. microplus* were capable to induce immunity against the ticks after bovine immunizations (Sugumar *et al*., 2011). Subsequently, several concealed antigens derived from *R. microplus* midgut were identified. The antigens Bm86, Bm91, Bm95 and BmPRM, were wide tested separately or together in immunization schedules, eliciting several degrees of immune protection (Marcelino *et al*., 2012).

The low protection degrees and efficacy variables obtained by Bm86 and Bm95 antigens in challenges against *R. microplus*, motivated the research for new immunogens capable of exclude the immune repertory induced by larger glycoproteins. Thus, based in the immunogenic epitopes of protein Bm86, were described the peptide SBm7462® by means of bioinformatics methodologies (Patarroyo *et al*., 2002; Peconick *et al*., 2008), which served as platform to recombinant production peptides by fermentation in *Pichia pastoris*.

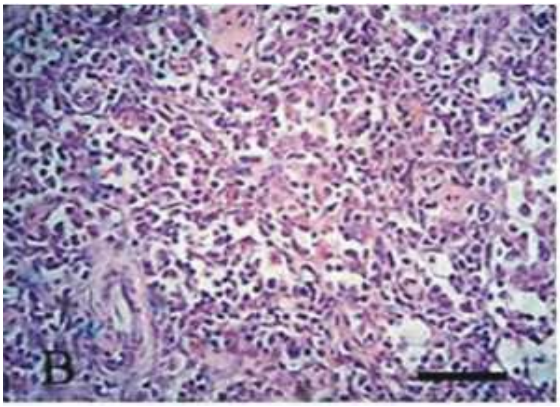
Moreover, the advances in immune response knowledge, recognized in a good peptide as vaccine candidate, the capacity to suitable processing into APCs and their ability to immunological synapse induction with lymphocytes, allowing T and B cells activation up to clonal amplification and memory induction in lymph nodes. To explain these premises, will be deal posteriorly the immune events triggered in assays with peptides as immunogens against *R. microplus* ticks.

1.2. Affinity Selection and Immune Protection with Peptides

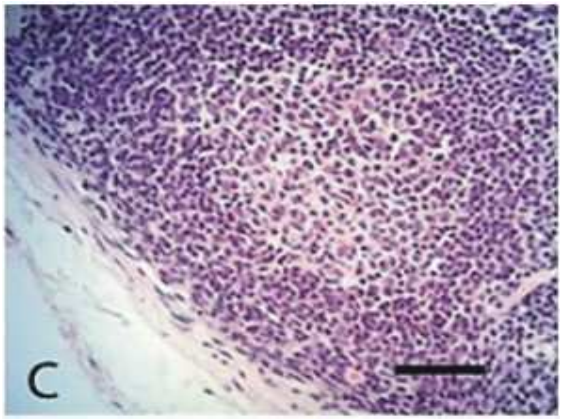
In the contex of immune respose with peptides, is wide known that Dendritic Cells (DCs) processed protein antigens in peptides that are loaded by major histocompatibility complex molecules class I and II (MHC class I and class II), being transported to the cell membrane for recognition by highly affinity T cells (Palucka *et al*., 2010). Likewise, it was reported that the peptide may interact directly with the MHC class II molecule on the surface of APCs, parallel to the lifetime of MHC class II molecule (Nene *et al*., 2012).



(A)



(B)



(C)

Fig. 1. Microphotography of bovine lymph nodes by H&E technique. (A) 5 days after second immunization with rSBm7462. (B) Details from medullary cords 15 days after second immunization with rSBm7462. (C) Non-inoculated control. CG: germinal center. Scale bar 50 µm

Moreover, during T-dependent responses, Germinal Centers (GCs) are important anatomic sites for the development of high affinity antibodies. Also, the formations of GCs are essential for triggering memory B cells. In this form, GCs are organized in the adaptive immune context, beginning from rapid clonal expansion of forming cells (Victora and Nussenzweig, 2012; Cyster, 2010). Likewise, tests with synthetic peptide called SBm7462® in bovine immunized with 2 mg plus 1.5mg saponin as adjuvant, at 7 days after the first immunization in lymph nodes histological sections, showed T cells zone hyperplasia with low CGs reaction, accompanied by slight hyperplasia of medullary region. At day 15, high follicles numbers were observed with GCs formation delimited by lymphocyte population, differentiated in dark and light regions. Meanwhile, the medullary cords hyperplasia was most intense 5 days after the second immunization, confirmed by IgG1 increased levels (Patarroyo *et al*., 2009).

However, when employing the recombinant peptide called rSBm7462, originated from the amino acids sequence of synthetic peptide, in bovine immunization, three times with interval of 28 days, with 2 mg of it peptide plus 1.5mg of saponin as adjuvant;fifth days after second immunization was observed CGs formation in lymph nodes, delimited by a lymphocyte population (**Fig. 1A**). Though, the hyperplasia in medullary cords was the most intense, 15 days after the second immunization (**Fig. 1B and C**) (Tafur, 2011). Similarly, IgGs levels showed a significant increase from the second immunization, reaching an increased level in the same period (Sousa, 2011).

These facts suggest poor immunological memory induction after the first immunization and sustain immune response increase after subsequent immunizations with booth peptides. To understand these immunological events, is important to detail the lymph nodes hyperplasia degrees observed in medullar region and gradual production of antibodies in experimental stages with anti-tick vaccines.

These events indicated the possible started proliferation of local B cells (blast), as a subsequence of interaction with T cells after first immunization whit boot peptides. Likewise, the activate B cells together high affinity T lymphocytes, may induce follicles cells migration to GCs formation. Later, it can trigger B cells differentiation from short-living Plasma cells (PBs) to long-term Plasma Cells (PCs). Thus, highly migratory PBs cells can move from outside the T cell area to medullary cords. In this local, PBs cells may differentiate into noncyclic PC cells, residing for several days before entering apoptosis.

However, in concordance with our observations, PBs cells started in GCs could induce extrafollicular way offering an initial wave of PCs (>3 days) and the follicular pathway provides sustained delayed wave of PC (>7 days), related whit previously mentioned facts. Then, cell migrating from the lymph node to bone marrow as to mucous membranes where maintained the immunological memory, issue associated with increase immune response after immunization boosters with anti-ticks peptides (Fooksman *et al*., 2010; Luther, 2010).

Meanwhile, the protection levels after challenge with *R. microplus* were superior when a synthetic peptide was used, reaching 81.05% of efficacy. Though, with the recombinant peptide it was obtained 72.40% of protection (Patarroyo *et al*., 2009; Sousa, 2011). When synthetic peptide was used it was possible to determine the gradual increase of lymphocytes B CD21+ and lymphocytes CD4+ after the first immunization. In the same manner, compared with IL-10 increased expression in cattle immunized with recombinant peptide, it may help to understand the changes in acquired immune response. Nonetheless, the differences in bovine acquired immune response may be influenced by genetics polymorphisms of bovine MHC, generating changes in acquired immune responses (Patarroyo *et al*., 2009; Macdonald *et al*., 2010; Fidelis *et al*., 2011; Nene *et al*., 2012).

Furthermore, in cattle immunized with synthetic peptide, peroxidase anti peroxidase (PAP)-positive cells in paracortical region (T-cell zone), CGs and medullary cords of lymph nodes were founded 7 days after the first immunization. Additionally, 5 days after the second immunization, strongly PAP-positive cells in medullary cords were observed (Patarroyo *et al*., 2009). The rapid emergence of PAP-positive cells in CGs indicated that the synthetic peptide was captured and retained by resident DC-like, transported through conduit network for initial activation of T-cells. This phenomenon was studied by Gonzalez *et al*. (2011) and Le Roux and Florence (2012), who showed that resident DC-like maintain interactions with basal membrane components of the reticular fibers, allowing close contact with conduit networks, consequently occurred an efficient capture of lower molecular weight molecules.

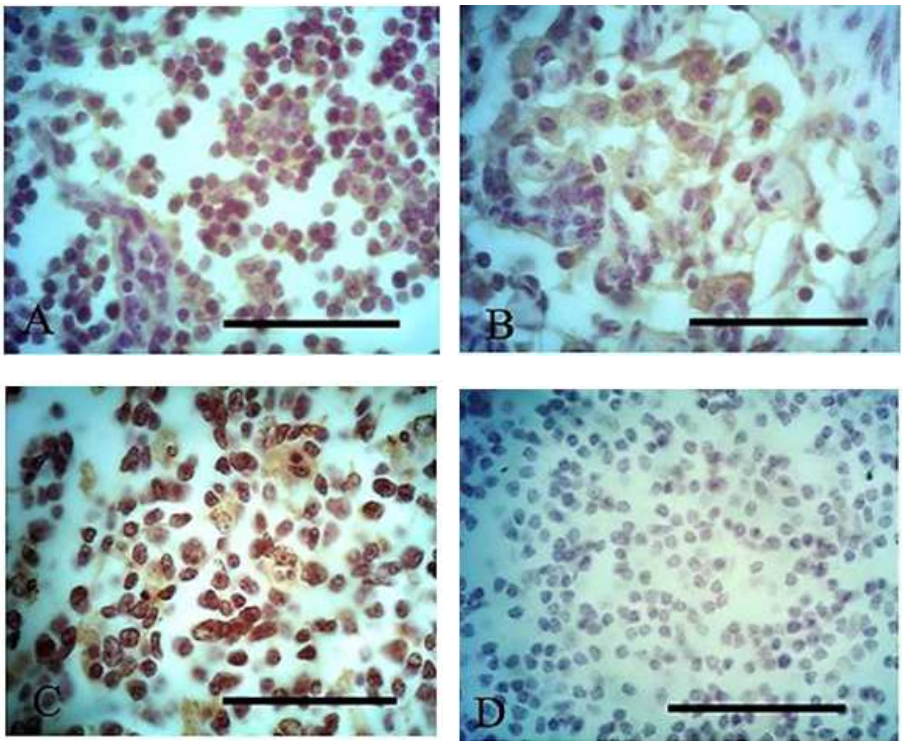


Fig. 2. Microphotography of bovine lymph nodes by PAP technique. (A) PAP-Positive cells in T zone 15 days day after first immunization with rSBm7462. (B) PAP-Positive cells in medullary cords 15 days after first immunization with rSBm7462. (C) PAP-Positive cells in CGs (D) Serum negative control. Scale bar 50 µm

However, when using recombinant peptide, PAP-positive cells were found in T-cell zone and medullary cords 15 days after the first immunization (**Fig. 2A and 2B**). Even though, PAP-positive cells in GCs were evident only 5 days after the second immunization, accompanied by highly PAP-positive cells in medullary cords (**Fig. 2C and D**) (Tafur, 2011).

The slow emergence of PAP-positive cells in CGs show that the DC-like maturation becomes essential for antigen presentation, suggesting that the recombinant peptide may have higher avidity to be recognized and processed by the DC-like, reaching the lymph nodes in strong association with these cells. So, it immune signal may become slow compared with conduit pathway in the immune events by synthetic peptides (Steinman, 2012).

Meanwhile, the PAP-positive cells founded in medullary cords after the second immunization by means of both peptides, was associated with the hyperplasia observed, suggesting an increase in clonal expansion, explaining the continuity of the immune response.

On the other hand, when used a second anti-tick recombinant peptide designed from immunogenic sequence of synthetic peptide, but with epitopes in tandem repeat (rSBm7462T) and using by immunization scheme described above, after 7 days of immunization with 2 mg of it peptide plus 1.5mg of saponin as adjuvant, were observed a rapid emergence of GCs in lymph nodes (**Fig. 3A**), accompanied by PAP-positive cells similar to observed with synthetic peptide (**Fig. 3B**). However, the apoptotic bodies with remarkable reduction of immune response were detected 5 days after the second immunization resulted in 52.72% of bovine protection after ticks challenge (**Fig. 3C**). These facts suggest that antigen processing pathway of recombinant peptides could change depending on the dose used and on antigen density. Issue treated by Jiskoot *et al*. (2012) when referenced that the immunogenicity of protein drugs was dependent of protein structure variability.

In this context, the peptide recombination system in *Pichia pastoris*, employed for anti-ticks peptides production, could induce glycosylation bridges N and O, changing the peptides conformation (De Schutter *et al*., 2009). Additionally, prediction analysis of protein expression indicated that the rSBm7462T peptide could have three *N*-glycosylation sites; whereas the peptide rSBm7462 could have one *N*-glycosylation site (Sossai, 2009).

Nonetheless, glycoproteins effect in APCs antigen processing is a little research area, its studies whit DCs revealed receptors expression which include Toll-Like Receptors (TLRs) and C-type Lectins Receptors (CLRs) (Osorio and Sousa, 2011).

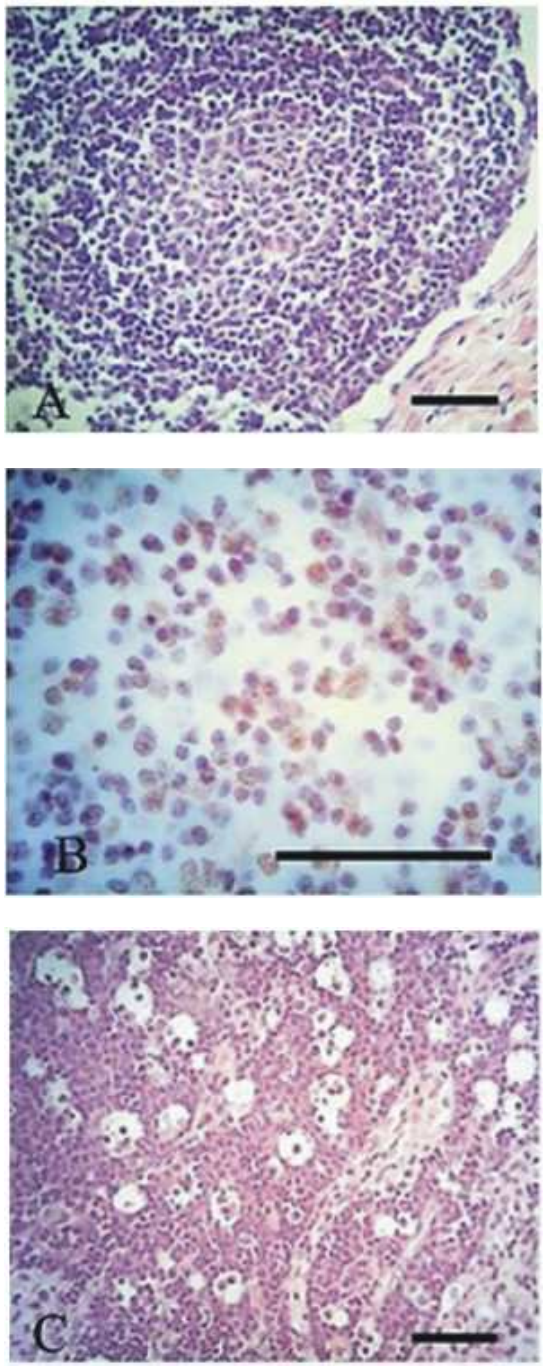


Fig. 3. Microphotography of bovine lymph nodes. (A) 7 days after first immunization with rSBm7462T by H&E technique. (B) PAP-Positive cells in GC 7 days after first immunization with rSBm7462T (C) Apoptotic bodies from medulla cords 5 days after second immunization with rSBm7462T. Scale bar 50 µm

The TLRs recognize Pathogens Associated Molecular Patterns (PAMPs), whereas CLRs recognizes pathogens carbohydrate profiles to intervening in DCs differentiation and migration process. Meanwhile, new assays confirmed an cross talk between CLR and TLR, these studies demonstrated an limited activation of TLR when ocurred the CLR activation (Geijtenbeek and Gringhuis, 2009). Additionally, the several degrees of glycosylation in recombinant anti-ticks peptides, suggests varied avidity degree for APCs peptide recognition via CLR activation, when associated with the differences observed in bovine immune response at first stage.

Moreover, the DC-like in immature state, express several CLRs which contain one or multiple Carbohydrate Recognition Domains (CRD) couplets to cell membrane, arranged to capture pathogens and MHC antigen processing (Sancho and Sousa, 2012). Furthermore, the fast and slow response started by anti-tick glycopeptides, suggests that bovine peripheral DC-like could recognize this peptide via CLRs, it elicits mechanisms that activates signals maturation of DC-like, bringing antigen processing and lymph nodes presentation through afferent lymph ducts, while immune signal it associated with glycosylation degrees.

However, the decreases in immune response with rSBm7462T peptide after the second immunization had a strong relationship with density and dose-response (phenomenon explained later). So, in relation with glycosylation sites and CLRs interaction, studies with DC-SING (CLR type II) based on lectin capacity to administer antigens in intracellular compartments and antigen presentation to naïve T cells, it demonstrated that molecule could have an important role in regulating immune tolerance. Similarly, tests whit Dectin-1, CLR responsible for yeast β-glucans recognition, showed that lectin can modulate the DCs for Th17 cells differentiation or IL 10 expansion to induce tolerogenic DCs. Also, the P-selectin coupling or DCs Immunoreceptor Expression (DCIR) stimulates inhibitory signals that limit DCs functionality. These events support the hypothesis that CLRs may activate immune control mechanisms in immunized bovine with glycoproteins in high dose schedule (Mascanfroni *et al*., 2011).

1.3. Doses and T Cell Priming

Previous studies elucidated that antigen high doses triggered T-cells suppression, through programmed cell death, after T cells activation by APCs. In this context, the interaction between high densities of APCs with low densities of T cells at high antigen doses induce apoptosis (Gabrysova *et al*., 2009). Posteriorly, studies focused on understanding the T cells priming in different peptide densities, showed that T cells proliferation with low peptides concentrations, associated with low interaction probability among T cells and antigen presented by APCs, similarly the encounters between naïve T cells and APCs in optimal antigen densities generated stable contacts and started rapid T cells proliferation (Bousso, 2008).

Considering the relationship between APCs antigen densities and interaction naïve T cells, studies to understand the activation signals released during T cells priming in lymph nodes were conducted. These experiments revealed that contacts occurred between naïve T cells-APC, exhibit markedly different durations and stabilities. Three prototypical interactions models were described: transitional interactions, long-term interactions and swarming (Davis, 2009; Hugues, 2010). However, in our experiments, structures that could confer immunity were observed, five days after second bovine immunization with rSBm7462 peptide, suggesting that lymph node clonal expansion by the use of this peptide could be the result from succeeding transient interactions between naïve T cells-APCs in well antigen densities.

To explain the low cell proliferation after the first immunization and subsequent cell proliferation by using succeeding immunizations with rSBm7462 peptide, previous experiments elucidated it during transient interactions, T cells do not stop completely, indeed they continue to roll on the APCs surface separated rapidly. If this process repeats by subsequent antigenic challenge, T cells could join in short interactions with APCs to trigger T cells proliferation. Moreover, transient interactions were significant to immune response mounting, followed by stable conjugates formation between T cells-APCs (Henrickson *et al*., 2008; Azar *et al*., 2010).

High antigenic densities with rSBm7462T peptide on APCs surface induced rapid T cells proliferation with GCs induction after the first immunization, resulting from possible stable contact emergence between T cells-APCs. Whereas, the low T cell proliferation observed with rSBm7462peptide, could result in transient interactions between T cells-APCs accompanied with low GCs reaction at the first stage; issue described by Henrickson *et al*. (2008) when confirmed that several antigen densities, induces different stimulus to GCs formation capable to altered T cell proliferation levels.

On the other hand, to elucidate the GCs and immune response reduction when using tandem peptide after bovine second immunization, it is necessary to analyze

the emergence of apoptotic bodies after second immunization with rSBm7462T. These fate, could result from cell death activation, which induces effector response interruption, influenced by antigens levels over superior limit capable to induce tolerance and cell proliferation decrease (Garrod *et al*., 2012). Similarly, in apoptosis made by high antigen doses, the T cell activation started signalization events including dowregulation of growth cytokine synthesis in cell cycle progression and TCR re-engagement (Azar *et al*., 2010). Meanwhile, Smith-Garvin *et al*. (2009) mentioned the inverse relationship existence between antigen dose and TCR affinity, enabling the low affinity TCR induction by high antigen dose and vice versa.

Additionally, in ruminants the γδ T cells may constitute up to 50-60% of the circulating T cells, these cells has antigen-presentation features similar in potency and efficacy to those seen in DCs (Price *et al*., 2010; Moser and Eberl, 2011). However, during immune response by synthetic peptide, was observed growth adaptive immune response after levels of γδ $WC1^+$ Tcell decrease in peripheral blood, suggesting that these cells may increase the APCs density in periperical tissues (Patarroyo *et al*., 2009). Likewise, the aumented of APCs densities with low T cells densities, could also contribute to apoptosis emergence when employed tandem peptide in high antigen dose (Garrod *et al*., 2012; Celly *et al*., 2012).

However, to understand the dominant immunity achieved in subsequent immunization by rSBm7462 peptide (third immunization), we thought that the immunity structures developed after second immunization with this, could result from transient interactions formed between T cells-APCs. Issue studied by Garrod *et al.* (2012) who observed transient interactions formation between T CD 8+ cells and DCs at low peptide concentrations, followed by stable contacts between themselves cells on same peptide dilution. Moreover, other studies showed that transient interactions were more relevant to beginning antigen response by formation of stable conjugates between T cells-DC with the ability to cell proliferation increase (Hugues, 2010; Azar *et al*., 2010).

1.4. Protein Aggregation and Immune Response

During recombinant anti-ticks immunogens expression analysis, it was suggested that larger amounts of hydrophobic interactions with rSBm7462T and lower from rSBm7462, indeed was described more cysteine residues for first peptide and scarce for second peptide (Sossai, 2009). Certainly, the cysteine disulfide bridges probably are the main chemical induction via of protein aggregation (Wang *et al*., 2012). Immunogenically has been associated with antigenicity increase, to enhancing T-dependent response by increasing the antigen binding to B cells receptors (Manning *et al*., 2010). This situation could be relationship with the rapid immune response observed after the first immunization by rSBm7462T, meanwhile it was evidenced a slow immune response with rSBm7462 at the first stage of bovine acquire immune response.

In concordance with CLR receptors previously inferred, aggregate recombinant anti-ticks peptides could be easily captured by APCs, inducing cell maturation and increased T-dependent response. Additionally, this aggregate peptide may generate cross-linking with B cells receptors to cells proliferation, leading the protein to lysosomal via downstream efficient T helper cells activation (Buttel *et al*., 2011; Wang *et al*., 2012). These situations contributed to explain the APCs maturation by experimental tests in relationship with PAP-positive cells levels.

2. CONCLUSION

Boot, bibliography relates and experimental assays whit anti-tick peptides, confirm the subunit vaccines ability to trigger mechanisms to adaptive immune response capable to induce, cellular, humoral and immunological protection. Though, concealed antigens during pathogenic process are not present to immune system, the findings described in this review demonstrate which synthetic and recombinant subunits antigens can previously qualify the bovine immune response to protect against ticks. The high recombinant densities anti-tick peptide at higher doses induces rapid maturation of APCs, activating greatest immune control inhibitory mechanisms by subsequent doses. Meanwhile, immunization with low recombinant densities anti-tick peptide in suitable doses can induce gradual maturation of APCs triggering better immune protection.

3. ACKNOWLEDGEMENTS

The reachers thank FAPEMIG (Minas Gerais State Research Foundation Brazil) for providing financial support for the projects. We thank Marcio Mendes for technical assistance, Aloizio Carlos da Silva for assistance with animals experiment, José de Oliveira for professional support, Vanessa Marinheiro for experiment assistance and Gabriela Santistevan for English redaction.

4. REFERENCES

Azar, G.A., F. Lemaitre, E.A. Robey and P. Bousso, 2010. Subcellular dynamics of T cell immunological synapses and kinapses in lymph nodes. Proc. Natl. Acad. Sci. USA., 107: 3675-3680. DOI: 10.1073/pnas.0905901107

Bousso, P., 2008. T-cell activation by dendritic cells in the lymph node: Lessons from the movies. Nat. Rev. Immunol., 8: 675-684. DOI: 10.1038/nri2379

Buttel, I.C., P. Chamberlain, Y. Chowers, F. Ehmann and A. Greinacher *et al*., 2011. Taking immunogenicity assessment of therapeutic proteins to the next level. Biologicals, 39: 100-109. DOI: 10.1016/j.biologicals.2011.01.006

Celly, S., M. Day, A.J. Muller, C. Molina-Paris and G. Lythe *et al*., 2012. How many dendritic cells are required to initiate a T-cell response? Blood, 120: 3945-3948. DOI: 10.1182/blood-2012-01-408260

Cyster, J.G., 2010. Shining a light on germinal center B cells. Cell, 143: 503-505. DOI: 10.1016/j.cell.2010.10.036

Davis, D.M., 2009. Mechanisms and functions for the duration of intercellular contacts made by lymphocytes. Nat. Rev. Immunol., 9: 543-555. DOI: 10.1038/nri2602

De Mestre, A.M., 2011. Functions of ectopically transplanted invasive horse trophoblast. Reproduction, 141: 849-856.

De Schutter, K., Y.C. Lin, P. Tiels, A.V. Hecke and S. Glinka *et al*., 2009. Genome sequence of the recombinant protein production host Pichia pastoris. Nat. Biotech, 27: 561-566. DOI: 10.1038/nbt.1544

Desmettre, P., 2011. Veterinary Vaccines in the Development of Vaccination and Vaccinology. In: History of Vaccine Development, Plotkin, A.S. and A. Stanley, (Edn), Springer, New York, NY, ISBN-10: 978-1-4419-1338-8, pp: 329-338.

Fidelis, C., L.S. De Araujo, G.A. Tafur and J.H. Patarroyo, 2011. Using RT-real time PCR to quantify the expression of genes associated with the immune response of cattle vaccinated with the recombinant antigen rBm7462. Biomedical, 31: 259-260.

Fooksman, D.R., T.A. Schwickert, G.D. Victora, M.L. Dustin and M.C. Nussenzweig *et al*., 2010. Development and migration of plasma cells in the mouse lymph node. Immunity, 33: 118-127. DOI: 10.1016/j.immuni.2010.06.015

Gabrysova, L., K.S. Nicolson, H.B. Streeter, J. Verhagen and C.A. Sabatos-Peyton *et al*., 2009. Negative feedback control of the autoimmune response through antigen-induced differentiation of IL-10-secreting Th1 cells. J. Exp. Med., 206: 1755-1767. DOI: 10.1084/jem.20082118

Garrod, K.R., H.D. Moreau, Z. Garcia, F. Lemaitre and I. Bouvier *et al*., 2012. Dissecting T cell contraction in vivo using a genetically encoded reporter of apoptosis. Cell Reports, 2: 1438-1447. DOI: 10.1016/j.celrep.2012.10.015

Geijtenbeek, T. and S. Gringhuis, 2009. Signalling through C-type lectin receptors: Shaping immune responses. Nat. Rev. Immunol., 7: 465-479. DOI: 10.1038/nri2569

Gonzalez, S.F., S.E. Degn, L.A. Pitcher, M. Woodruff and B.A. Heesters *et al*., 2011. Trafficking of B cell antigen in lymph nodes. Annu. Rev. Immunol., 29: 215-233. DOI: 10.1146/annurev-immunol-031210-101255

Grimm, S.K. and M.E. Ackerman, 2013. Vaccine design: Emerging concepts and renewed optimism. Curr. Opin. Biotech., 2013: 1-11. DOI: 10.1016/j.copbio.2013.02.015

Henrickson, S.E., T.R. Mempel, I.B. Mazo, B. Liu and M.N. Artyomov *et al*., 2008. T cell sensing of antigen dose governs interactive behavior with dendritic cells and sets a threshold for T cell activation. Nat. Immunol., 9: 282-291. DOI: 10.1038/ni1559

Hugues, S., 2010. Dynamics of dendritic cell-T cell interactions: A role in T cell outcome. Semin Immunopathol., 32: 227-238. DOI: 10.1007/s00281-010-0211-2

Jiskoot, W., T.W. Randolph, D.B. Volkin, C.R. Middaugh and C. Schoneich *et al*., 2012. Protein instability and immunogenicity: Roadblocks to clinical application of injectable protein delivery systems for sustained release. J. Pharma Sci., 101: 946-954. DOI: 10.1002/jps.23018

Le Roux, D. and N. Florence, 2012. New insights into antigen encounter by B cells. Immunobiology, 217: 1285-1291. DOI: 10.1016/j.imbio.2012.07.011

Luther, S.A., 2010. Plasma cell precursors: Long-distance travelers looking for a home. Immunity, 33: 9-11. DOI: 10.1016/j.immuni.2010.07.010

Macdonald, I.K., M. Harkiolaki, L. Hunt, T. Connelley and A.V. Carroll *et al*., 2010. MHC class I bound to an immunodominant *Theileria parva* epitope demonstrates unconventional presentation to T cell receptors. PLoS Pathogens, 6: e1001149-e1001149. DOI: 10.1371/journal.ppat.1001149

Manning, M.C., K.D. Chou, B.M. Murphy, R.W. Payne and D.S. Katayama, 2010. Stability of protein pharmaceuticals: An update. Pharma Res., 27: 544-575. DOI: 10.1007/s11095-009-0045-6

Marcelino, I., A.M., de Almeida, M. Ventosa, L. Pruneau and D.F. Meyer *et al*., 2012. Tick-borne diseases in cattle: Applications of proteomics to develop new generation vaccines. J. Prot, 75: 4232-42350. DOI: 10.1016/j.jprot.2012.03.026

Mascanfroni, I.D., J.P. Cerliani, S. Dergan-Dylon, D.O. Croci and J.M. Ilarregui, 2011. Endogenous lectins shape the function of dendritic cells and tailor adaptive immunity: Mechanisms and biomedical applications. Int. Immunopharmacol., 11: 833-841. DOI: 10.1016/j.intimp.2011.01.021

Moser, B. and M. Eberl, 2011. γδ T-APCs: A novel tool for immunotherapy?. Cell Mol. Life Sci., 68: 2443-2452. DOI: 10.1007/s00018-011-0706-6

Nene, V., N. Svitek, P. Toye, W.T. Golde and J. Barlow *et al*., 2012. Designing bovine T cell vaccines via reverse immunology. Ticks Tick-Borne Dis., 3: 188-192. DOI: 10.1016/j.ttbdis.2011.12.001

Neto, D.F.D.L., C.W. Arns, D.R. Stach-Machado, F.R. Spilki and J. Mattoso *et al*., 2013. Epitope mapping of tobacco mosaic virus capsid protein: Prediction and experimental data from spot synthesis. Mol. Biol., 2: 1-10.

Oliveira, D., 2006. Avaliação da cinética leucocitaria sanguínea em bovinos após a aplicação simultânea dos imunógenos sintéticos anti-*babesia bovis* (SBbo 23290) e anti- *Rhipicephalus (Boophilus) microplus* (SBm7462). Unpublished Thesis in Partial Fulfilment of the Requirements for the Degree of Master Science, Federal Viçosa University, Viçosa, Minas Gerais, Brasil.

Osorio, F. and C.R.E. Sousa, 2011. Myeloid c-type lectin receptors in pathogen recognition and host defense. Immunity, 34: 651-664. DOI: 10.1016/j.immuni.2011.05.001

Palucka, K., J. Banchereau and I. Mellman, 2010. Designing vaccines based on biology of human dendritic cell subsets. Immunity, 33: 464-478. DOI: 10.1016/j.immuni.2010.10.007

Patarroyo, J.H., M.I. Vargas, C.Z. Gonzalez, F. Guzman and O. Martins-Filho *et al*., 2009. Immune response of bovines stimulated by synthetic vaccine SBm7462 against *Rhipicephalus* (*Boophilus*) *microplus*. Vet. Parasitol., 166: 333-339. DOI: 10.1016/j.vetpar.2009.09.036

Patarroyo, J.H., R.W. Portela, R.O. De Castro, J.C. Pimentel and F. Guzman *et al*., 2002. Immunization of cattle with synthetic peptides derived from the *Boophilus microplus* gut protein (Bm86). Vet. Immunol. Immunopathol., 88: 163-172. DOI: 10.1016/S0165-2427(02)00154-X

Peconick, A.P, S. Sossai, F.A. M.Q.R.B. Girao and C.H Rodrigues *et al*., 2008. Synthetic vaccine (SBm7462) against the cattle tick *Rhipicephalus (Boophilus) microplus*: Preservation of immunogenic determinants in different strains from South America. Exp. Parasitol., 119: 37-43. DOI: 10.1016/j.exppara.2007.12.007

Plotkin, S. and S. L. Plotkin, 2011. The development of vaccines: How the past led to the future. Nat. Rev. Microbiol., 9: 889-893. DOI: 10.1038/nrmicro2668

Price, S., M. Davies, B. Villarreal-Ramos and J. Hope, 2010. Differential distribution of WC1(+) gammadelta TCR(+) T lymphocyte subsets within lymphoid tissues of the head and respiratory tract and effects of intranasal M. bovis BCG vaccination. Vet. Immunol. Immunopathol., 136: 133-137. DOI: 10.1016/j.vetimm.2010.02.010

Sancho, D. and C.R.E. Sousa, 2012. Signaling by myeloid C-type lectin receptors in immunity and homeostasis. Annu. Rev. Immunol., 30: 491-529. DOI: 10.1146/annurev-immunol-031210-101352

Seib, K.L., X. Zhao and R. Rappuoli, 2012. Developing vaccines in the era of genomics: A decade of reverse vaccinology. Clin. Microbiol. Inform., 18: 109-116. DOI: 10.1111/j.1469-0691.2012.03939.x

Sette, A. and R. Rappuoli, 2010. Reverse vaccinology: Developing vaccines in the era of genomics. Immunity, 33: 530-441. DOI: 10.1016/j.immuni.2010.09.017

Smith-Garvin, J.E., G.A. Koretzky and M. S. Jordan, 2009. T cell activation. Annu. Rev. Immunol., 27: 591-619. DOI: 10.1146/annurev.immunol.021908.132706

Sossai, S., 2009. Expressão de quatro peptídeos recombinantes derivados do peptídeo sintético sbm7462 em *Pichia pastoris* e avaliação da resposta imunológica induzida em camundongos. Unpublished Dissertation in Partial Fulfilment of the Requirements for the Degree of Doctor Science, Federal Viçosa University, Viçosa, Minas Gerais, Brasil.

Sousa, N.D.E., 2011. Avaliação da eficacia das vacinas recombinantes rbm7462-seq1 e rbm7462-seq4 contra o carrapato *Rhipicephalus microplus*. Unpublished Thesis in Partial Fulfilment of the Requirements for the Degree of Master Science, Viçosa Federal University, Viçosa, Minas Gerais, Brazil.

Steinman, R., 2012. Decisions about dendritic cells: Past, present and future. Annu. Rev. Immunol., 30: 1-22. DOI: 10.1146/annurev-immunol-100311-102839

Strugnell, R., F. Zepp, A. Cunningham and T. Tantawichien, 2011. Vaccine antigens. Persp Vac., 1: 61-88. DOI: 10.1016/j.pervac.2011.05.003

Sugumar, P., D. Chandran, R.G. Sudha, P.V. Shahana and D.K. Maske *et al*., 2011. Recombinant mid gut antigen (Bm95) as a vaccine against Indian *Rhiphicephalus haemaphysaloides* in *Bos indicus* cattle. Res. Vet. Sci., 90: 262-268. DOI: 10.1016/j.rvsc.2010.05.037

Tafur, G.A., 2011. Imunização de bovinos com dois peptídeos recombinantes derivados do peptídeo sbm7462®: Resposta imune de linfonodos e alterações histologicas do intestino do *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887). Unpublished thesis in partial fulfilment of the requirements for the degree of Master Science, Federal Viçosa University, Viçosa, Minas Gerais, Brasil.

Victora, G.D. and M.C. Nussenzweig, 2012. Germinal centers. Annu. Rev. Immunol., 30: 429-457. DOI: 10.1146/annurev-immunol-020711-075032

Wang, W., S.K. Singh, N. Li, M.R. Toler and K.R. King *et al*., 2012. Immunogenicity of protein aggregates--concerns and realities. Int. J. Phar., 431: 1-11. DOI: 10.1016/j.ijpharm. 2012.04.040