

## Potential mechanism of action of J5 vaccine in protection against severe bovine coliform mastitis

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(Received 15 May 2001; accepted 14 September 2001)

**Abstract** – Coliform mastitis is one of the most difficult diseases to treat in the modern dairy industry. Curative therapy with antibiotics remains only moderately effective and depends on the stage at which the disease is treated. The most successful strategies for combating coliform mastitis appear to be prevention by hygienic management or prophylactic immunization. The severity of clinical symptoms of coliform mastitis has been shown to be reduced by immunization with the *Escherichia coli* J5 vaccine. However, although the J5 vaccine has been licensed in the United States for about 10 years, the immunological basis of its mechanism of action is still unknown. Until now, protection by J5 vaccination has often been explained by a straightforward mechanism of enhanced antibody production resulting in increased opsonization of coliform bacteria and lipopolysaccharides (LPS). The possibility that J5 vaccination could decrease risk factors for coliform mastitis such as impaired blood polymorphonuclear neutrophil leukocyte (PMN) diapedesis has never been investigated. This review provides arguments to support the hypothesis that J5 vaccination may reduce the severity of coliform mastitis by inducing a condition of mammary gland hyper-responsiveness, characterized by a T helper 1 (Th1) response and mediated by memory cells inside the mammary gland, finally resulting in enhanced PMN diapedesis upon an intramammary infection.

**dairy cow / coliform mastitis / J5 vaccine / T cell / immune response**

**Résumé** – Mécanismes d'action possibles du vaccin J5 dans la protection contre la mammite coliforme sévère de la vache laitière. La mammite coliforme est une des maladies les plus difficiles à traiter dans l'industrie laitière moderne. La thérapie curative avec des antibiotiques reste peu efficace, dépendant du stade de la maladie. Les stratégies les plus efficaces pour combattre la mammite semblent être la prévention par des mesures d'hygiène ou la prophylaxie vaccinale. La sévérité des symptômes cliniques de la mammite coliforme peut être réduite par immunisation avec le vaccin *Escherichia coli* J5. Cependant, alors que le vaccin J5 a été mis sur le marché aux États-Unis depuis

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environ 10 ans, les bases immunologiques de son mécanisme d’action ne sont pas connues. Jusqu’à présent, la protection par le vaccin J5 a souvent été attribuée à un simple mécanisme de stimulation de la production d’anticorps résultant en une augmentation de l’opsonization des bactéries coliformes et des lipopolysaccharides (LPS). La possibilité que la vaccination avec J5 pourrait diminuer les facteurs de risque de la mammite coliforme, tels qu’une diapédèse insuffisante des leucocytes neutrophiles polymorphonucléaires (PMN) sanguins, n’a jamais été étudiée. Cet article de synthèse cite des arguments supportant l’hypothèse que la vaccination avec J5 pourrait réduire la sévérité de la mammite coliforme en induisant une hyper-sensibilité de la glande mammaire. Celle-ci serait caractérisée par une réponse immunitaire de type “T helper 1” par l’entremise de cellules mémoire de la glande mammaire, résultant finalement dans une augmentation de la diapédèse de PMN suivant une infection intramammaire.

**vache laitière / mammite coliforme / vaccin J5 / cellule T / réponse immunitaire**

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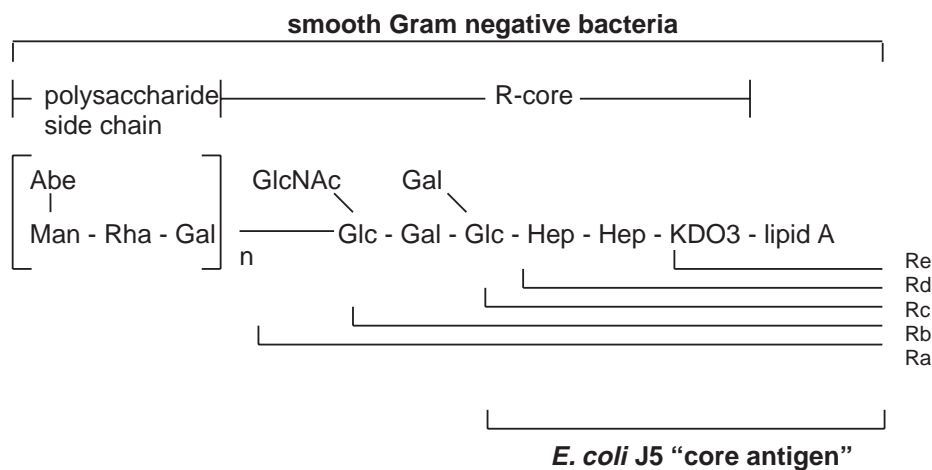
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## 1. INTRODUCTION

The incidence of severe bovine mastitis caused by environmental bacteria such as *Escherichia coli* is quite high during the early postpartum period. A great individual variability between cows in biological responses to intramammary infections is observed during this period. In fact, all degrees of severity from peracute to subclinical are possible [54]. The susceptibility of individual cows to severe coliform mastitis appears to be associated with the degree of neutropenia or impairment of blood polymorphonuclear neutrophil leukocyte (PMN) function. The most important risk factors for severe *E. coli* mastitis are slow migration of blood PMN into the mammary gland [14, 23, 55] and their impaired oxidative burst activity [1, 6, 13]. Lymphocyte function such as proliferation and antibody response has also been found to be impaired during early lactation [30],

but a relation with the severity of coliform mastitis has not been clearly established.

The *Escherichia coli* J5 vaccine has been successfully used in the protection against severe coliform mastitis [10, 21]. *Escherichia coli* J5 is a rough (Rc) mutant *E. coli* strain. Upon J5 vaccination, antibodies are therefore generated against the core antigen, which consists of lipid A and some common core polysaccharides (2-keto-3-deoxyoctonate, heptose and glucose residues) (Fig. 1) [4, 53]. The presence of core antigen compounds only, which – in contrast with O antigens – are highly conserved among Gram negative bacteria, forms the basis of cross-protection against a wide range of Gram negative bacteria. At first sight, protection afforded by J5 vaccination appears to be related to increased specific antibody production against LPS core antigens and enhanced opsonization of bacteria. However, although serum and milk antibody titers are increased as a result



**Figure 1.** Lipopolysaccharide (LPS) structure of smooth and rough Gram negative bacteria. The LPS structure of smooth bacteria consists of a polysaccharide side chain (O antigen), R-core polysaccharides and lipid A. The LPS structure of *Escherichia coli* J5 (a rough Rc mutant) lacks the O antigen and consists only of lipid A and some common core polysaccharides (2-keto-3-deoxyoctonate, 2 heptose and 1 glucose residues). Together, this LPS structure is called the "core antigen". Abe, abequose; Man, mannose; Rha, Rhamnose; Gal, galactose; GlcNAc, N-acetylglucosamine; Glc, glucose; Hep, Heptose; KDO, 2-keto-3-deoxyoctonate (after [53]).

of vaccination [18, 19], a proportionally decreased incidence and severity of *E. coli* mastitis has not always been readily apparent [15, 21]. There is practically no information available about the effects of J5 vaccination on risk factors for severe coliform mastitis such as decreased PMN number and function. From the important role of PMN in the pathogenesis of coliform mastitis as described above, it can be proposed that the mechanism of action of J5 vaccination should be based on the re-establishment or compensation for reduced PMN functions in order to reduce severity during coliform mastitis.

## 2. POTENTIAL MECHANISMS OF ACTION OF THE J5 VACCINE

Mastitis research has been concentrated on the role of PMN as a defense mechanism by itself; less attention has been paid on the characterization of lymphocyte-mediated mechanisms activating these cells. Several recent studies [29, 44] suggest that lymphocytes could also play an important role, but their precise function in the pathogenesis of coliform mastitis remains unclear. Two major pathways for a role of lymphocytes in the mechanism of action of J5 vaccination are explored: (1) modulation of the T cell mediated immune response; and (2) opsonizing or neutralizing effects of enhanced serum and/or milk antibody titers.

### 2.1. General schedule of events during J5 vaccination

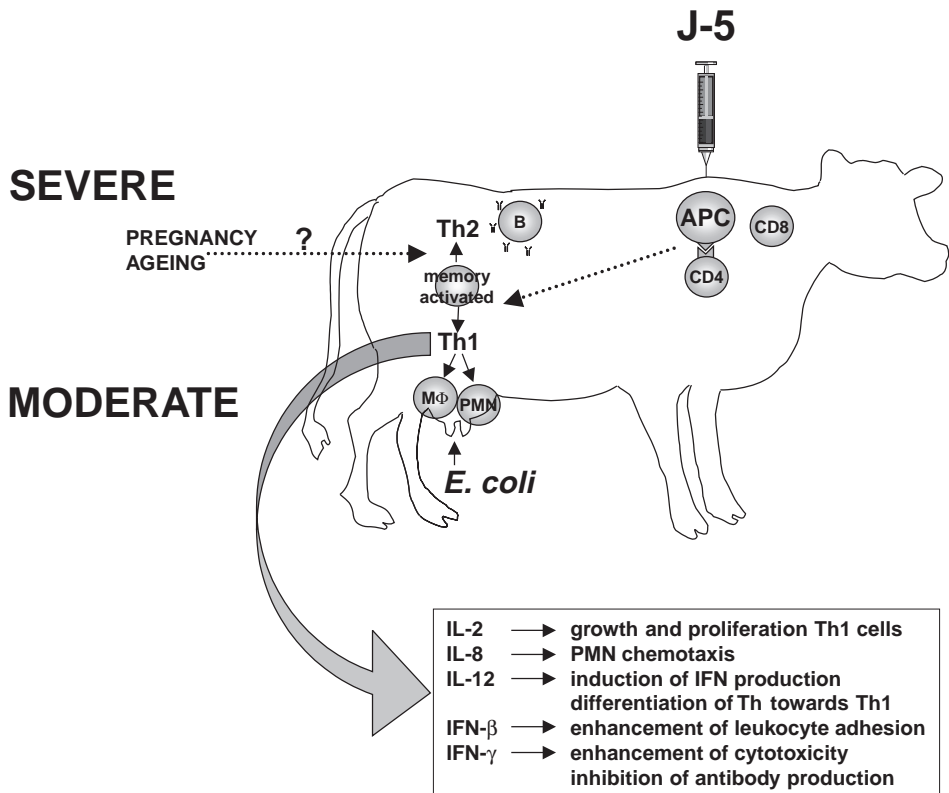
The events that will theoretically occur during J5 vaccination can be derived from human and mouse models (Fig. 2) (reviewed by Guidry and O'Brien [12]). Upon subcutaneous vaccination, the vaccine first binds to resident antigen-presenting cells (APC) which can be macrophages, activated B cells or dendritic cells. After antigen processing, the resulting products are

expressed on the membrane surface in conjunction with major histocompatibility class II (MHCII) molecules. Antigen-presenting cells migrate to lymphoid tissues, where they bind to CD4<sup>+</sup> T cells (T helper cells, Th). Both CD4<sup>+</sup> and CD8<sup>+</sup> (cytotoxic, Tc or suppressor T cells, Ts) belong to the class of  $\alpha\beta$ T cells. These cells can develop memory, resulting in a faster and more pronounced response after a 2nd or 3rd contact. Milk lymphocytes of healthy cows indeed display the phenotype of memory/activated cells [39, 48].

### 2.2. Hypothesis 1: J5 vaccination could polarize the inflammatory response during coliform mastitis

#### 2.2.1. General characteristics of T helper type 1 and 2 responses

Upon binding of APC with CD4<sup>+</sup> T cells, the immune response can be classified as a Th1 or Th2 response. Th1 responses are characterized by activation of macrophages and PMN adhesion, diapedesis and opsonization. Typical Th1 cytokines are interleukin-2 (IL-2, growth and proliferation of Th1 lymphocytes), IL-8 (stimulation of PMN chemotaxis and induction of endothelial binding sites on PMN), IL-12 (induction of interferon (IFN) production), IFN- $\beta$  (stimulation of leukocyte adhesion) and IFN- $\gamma$  (stimulation of cytotoxicity and inhibition of antibody production). A typical Th2 response consists of B cell activation to proliferate and differentiate into antibody-producing plasma cells. Th2 cytokines are IL-4 (stimulation of maturation and Ig class switching), IL-5 (stimulation of B cell proliferation and differentiation) and IL-10 (inhibition of cell-mediated immunity) (reviewed by Guidry and O'Brien [12]). From these data it can be proposed that a Th1 response would be beneficial in the outcome of *E. coli* mastitis, because of its stimulatory effects on PMN diapedesis.



**Figure 2.** Potential mechanism of action of the J5 vaccine in reducing severity of coliform mastitis in dairy cows. *Escherichia coli* J5 first binds to antigen-presenting cells (APC) which can be macrophages, activated B cells or dendritic cells (DC). After antigen processing, the product is expressed on the membrane surface in conjunction with major histocompatibility class II (MHCII) molecules. APC migrate to lymphoid tissues, where they bind to CD4<sup>+</sup> T cells (T helper cells, Th). Both CD4<sup>+</sup> T cells and DC can develop memory, so that the response upon intramammary coliform infections in J5-vaccinated cows will be faster and more pronounced than in non-vaccinated cows. CD4<sup>+</sup> T cells and DC can migrate towards the mammary gland. During coliform mastitis, APC binding with CD4<sup>+</sup> T cells can result in a Th1 or Th2 response. Th1 responses are characterized by activation of macrophages and PMN adhesion, diapedesis and opsonization whereas a typical Th2 response consists of B cell proliferation and differentiation. From these data it is hypothesized that a Th1 response is preferentially mounted in J5-vaccinated cows, because this would benefit the outcome of *E. coli* mastitis. This could be mediated by memory CD4<sup>+</sup> T cells and/or DC1 cells within the mammary gland. The inability to mount a Th1 response in severely diseased cows during coliform mastitis could be tentatively attributed to pregnancy and/or ageing.

### 2.2.2. T helper type 1 immune response and delayed type hypersensitivity

A typical moderate response during coliform mastitis is consistent with the characteristics of a Th1 type response: enhanced

PMN migration, diapedesis and activation (Fig. 2). This hypothesis is supported by the positive effects of IFN-γ treatment on the outcome of *E. coli* mastitis [47]. Delayed type hypersensitivity (DTH) reactions are also part of the Th1 response and may be

induced by J5 vaccination. Preliminary evidence for the existence of DTH reactions in the bovine mammary gland comes from an experiment in which enhanced PMN diapedesis into the mammary gland was induced after intramammary ovalbumin (OA) injection in subcutaneously OA vaccinated cows [5]. These results suggested that an antigen-specific pathway could be elicited in the mammary gland upon subcutaneous immunization with that specific antigen. This could also be the case during J5 vaccination, because PMN infiltration into the mammary gland has been enhanced in vaccinated cows [19]. Similar findings have recently been obtained with *Staphylococcus aureus*  $\alpha$ -toxin immunized cows [39].

### **2.2.3. Cell- or antibody-mediated types of hypersensitivity reactions?**

The etiology of hypersensitivity is complex: even in human medicine hypersensitivity reactions are difficult to classify as antibody- or cell-mediated. Because the effects seen in subcutaneously OA-vaccinated cows could not be mimicked by intramammary infusion of antigen-antibody complexes [5], it seems likely that possible hypersensitivity reactions during J5 vaccination are cell- and not antibody-mediated. This hypothesis is supported by the observation that treatment of mice [11] and neonatal calves [31] with J5 antiserum did not protect them against shock during intravenous LPS injection. These observations support the hypothesis of a cell-mediated pathway during J5 vaccination, rather than passive protection through increased serum or milk antibody titers.

### **2.2.4. Underlying mechanism of failure to mount a Th1 response**

#### **2.2.4.1. Lymphocyte phenotypes during the early postpartum period**

Failure of the immune response in dairy cows during coliform mastitis could

be associated with changes in the phenotype of blood mononuclear cells during the periparturient period. A dramatic decrease of some blood T cell subsets were observed shortly after parturition, whereas B cells were unchanged [24]. During early lactation, a decreased CD4<sup>+</sup> to CD8<sup>+</sup> milk lymphocyte ratio was found [33]. Kimura et al. [22] reported a significant decrease of blood CD3<sup>+</sup>, CD4<sup>+</sup> and  $\gamma\delta$ T cells at parturition. Decreased amounts of Th cells have indeed been used as indicators for an impaired immune function.

#### **2.2.4.2. Role of pregnancy and ageing in the polarization of the immune response**

Pregnancy has been proposed to cause the maternal immune system to preferentially mount a Th2 response [58] (Fig. 2). As a Th1 response may result in fetal absorption, the Th1 cytokines are generally harmful to the maintenance of pregnancy. Bovine blood CD4<sup>+</sup> lymphocytes have indeed been shown to function primarily as Th2 effector cells during the postpartum period [44]. It appears that Th2 cytokines are predominantly produced by cells from the fetoplacental unit in humans. Vaccination with paternal lymphocytes results in a shift towards a Th2 response and is associated with successful pregnancy in patients with recurrent spontaneous abortions [42]. In dairy cows, modulation of PMN function by placenta-derived glycoproteins has been found [7, 16], but their role in immune modulation during coliform mastitis is not completely understood. The phenomenon that pregnancy can modulate the immune response towards Th2 could therefore explain the decreased capacity to mount an adequate Th1 response during coliform mastitis in early postpartum dairy cows. Malfunction of the immune system in the elderly also appears to be associated with a Th1 to Th2 cytokine production shift [9]. Increased susceptibility to severe coliform mastitis has also been observed in older cows [55] (Fig. 2). Together, these findings

suggest that impairment of the immune system in periparturient and in older cows could also be associated with a shift in cytokine synthesis during coliform mastitis.

### **2.2.5. Role of memory cells in the mammary gland**

The inflammatory response upon intramammary challenge could be accelerated if memory cells were present inside the mammary gland. These memory or activated cells could be T lymphocytes or dendritic cells that have specifically migrated (homing) from local subcutaneous lymph nodes at the site of J5 vaccination towards the mammary gland (Fig. 2).

#### **2.2.5.1. Homing of memory T cells towards the mammary gland**

The homing phenomenon in general and to the mammary gland in particular is poorly characterized in dairy cows. A hypothetical pathway can be derived from human, rat or sheep models. Migration of memory T cells occurs preferably through non-lymphoid tissues whereas naïve T cells migrate to lymph nodes. The presence of lymphocytes in tissues is part of mucosal immunity. These tissues are called “mucosa associated lymphoid tissues (MALT)”. MALT is structurally characterized by follicle-associated lymphoepithelium and the presence of secondary follicles [3]. It can also be immunohistochemically identified by the presence of migrating activated lymphocytes and IgA producing plasma cells [2]. Typical MALT are the gut, lungs, nose (rodents), mesenteric lymph nodes, skin and subcutaneous lymph nodes (sheep) and the mammary gland (pigs). In cows, the presence of MALT has been described in the intestine [34] and in tonsils [36]. Histological evidence for MALT inside the bovine mammary gland tissue is not available, but plasma cells have been demonstrated in mammary gland mucosa, with increasing concentrations from milk-secreting

parenchyma to the distal teat end mucosa [32]. The local production of IgA, IgM and IgG2 in mammary tissue has also been demonstrated [35].

Homing of lymphocytes is controlled by the expression of homing receptors on lymphocytes and vascular addressins in the venules of the lamina propria of the gut and of the lactating mammary gland [59]. Tissue-specific T cell migration is predominantly a characteristic of  $\alpha\beta$ T cells ( $CD4^+$  and  $CD8^+$  cells), whereas  $\gamma\delta$ T cells migrate randomly [57]. More recent studies in sheep have shown that tissue-specific migration is restricted to  $CD4^+$  memory cells [28]. The number of lymphocytes in the porcine mammary gland increases from day 80 of pregnancy until parturition, under the influence of increased prolactin receptor density [40]. This suggests that homing towards the mammary gland may also occur through hormonal alterations in dairy cows. Actual data on bovine lymphocyte homing are, however, scarce. Milk lymphocytes display the phenotype of memory cells [39, 48]. The percentage of memory cells in milk was up-regulated following subcutaneous *S. aureus*  $\alpha$ -toxin immunization [39]. Although these authors used a different model than J5 vaccination, their findings support the general concept that memory T cells can specifically migrate towards the mammary gland in immunized cows.

#### **2.2.5.2. Milk lymphocyte phenotypes during mastitis**

During *S. aureus*  $\alpha$ -toxin induced mastitis,  $CD8^+$  cells were recruited into milk earlier than  $CD4^+$  cells: 12 h and 96 h after challenge, respectively [39]. This suggests an important role of  $CD8^+$  cells as effector cells in intramammary defense of immunized cows. In field cases, the  $CD4^+$  to  $CD8^+$  ratio of milk cells was increased during staphylococcal mastitis whereas it remained constant during streptococcal mastitis [46]. From these data it is clear that

changes in proliferation and trafficking of CD4<sup>+</sup> and CD8<sup>+</sup> are dependent on the antigenic stimulus and that lymphocytes probably play a crucial role in regulating the inflammatory response of the bovine mammary gland. The effect of J5 vaccination on the phenotype of milk lymphocytes in healthy cows and their response upon intramammary infections with coliform bacteria is presently unknown.

### **2.2.6. Antigen-presenting cell requirements for Th1 differentiation**

The APC requirements for differentiation of naïve CD4<sup>+</sup> cells into Th1 cells in J5-vaccinated cows are presently unknown. Both macrophages and dendritic cells but not B cells were found to play a role in the induction of Th1 development in mice [27]. Findings in humans also suggest that monocyte-derived dendritic cells (DC) play an important role in the generation of a polarized immune response. Committed DC are currently used for vaccination in cancer patients in order to induce a Th1-dominant immune response and stimulate a DTH reaction, which is required for the induction of tumor regression [38]. Optimal activation of T lymphocytes indeed depends on T cell receptor interaction with peptide/MHC complexes in conjunction with co-stimulatory signals, delivered by APC. Dendritic cells are widely distributed in tissues and play a major role in the induction of primary T cell dependent immune responses [37]. The Th1/Th2 commitment of T cells appears to occur within clusters of DC and T cells in T cell areas of lymphoid tissues where DC1 induce the differentiation of Th1 cells and DC2 induce Th2 cells. This commitment is controlled by typical Th1 and Th2 cytokines, respectively [43]. The role of DC in the pathogenesis of coliform mastitis and in the modulation of the immune response in J5-vaccinated cows currently remains unknown.

## **2.3. Hypothesis 2: J5 vaccination protects against severe coliform mastitis through increased serum and milk antibody titers**

### **2.3.1. Effect of J5 vaccination on serum and milk antibody titers**

The classical hypothesis for a mechanism of action of J5 vaccination consists in the protection by enhanced opsonization of LPS and bacteria by increased serum and milk antibody titers. Immunization with the J5 vaccine indeed increases antibody titers to *E. coli* J5 core antigens in serum and mammary secretions [50]. In a field study, high serum IgG1 titers against *E. coli* O111: B4 (J5) were associated with a decreased incidence of clinical coliform mastitis [51]. In addition, serum from J5 vaccinated cows showed enhanced in vitro opsonic activity for *E. coli* [17]. Thus, it can be hypothesized that J5 vaccination may protect against severe coliform mastitis by stimulation of specific antibody production against the common core antigen of Gram-negative bacteria, resulting in enhanced opsonization of bacteria and LPS. However, although increased serum and milk specific antibody titers are generally observed following J5 vaccination, a relation with the incidence or severity of mastitis has not always been established [15, 50]. Further evidence to reject the hypothesis that increased antibody titers provide protection in J5-vaccinated cows, comes from the conclusion of Hill [14] that opsonins are not a limiting factor in the pathogenesis of coliform mastitis. A fast PMN diapedesis and the availability of a high number of active PMN inside the infected mammary gland are much more critical for the outcome of severe coliform mastitis [14, 23, 54, 55].

### **2.3.2. Effect of lactation stage on antibody response**

Variability in antibody response to J5 immunization could be the result of variable

immunosuppression in early lactation dairy cows. For example, serum IgG2 titers recognizing *E. coli* J5 common core antigen following vaccination showed a coefficient of variation of 64% [15]. Serum antibody titers in J5 vaccinated cows are generally higher than in control cows, but significant effects of lactation stage on milk and serum IgG and IgM titers have been observed in both control and J5 vaccinated cows [18, 21, 45, 49]. Furthermore, periparturient cows can be phenotypically classified as low or high responders to immunization, based on the magnitude and kinetics of the antibody response to *E. coli* J5 or ovalbumin injection [29, 56]. Whereas about one third of the cows responded normally, two third showed a variable degree of hyporesponsiveness to peripartum immunization [29, 56]. These findings suggest that the immune deficiency which makes cows susceptible to *E. coli* mastitis may therefore also impair antibody response to active immunization during early lactation.

### **2.3.3. Effect of adjuvant and administration route on antibody response**

The antibody response to J5 immunization appears to be dependent on the adjuvant and the route of administration. In vaccinated neonatal calves, a significant increase in antibody titers was only observed with Freund incomplete adjuvant [52]. The route of immunization also appears to play a role in the antibody response. A vaccination schedule that included intramammary immunization between 2 subcutaneous immunizations resulted in higher serum antibody titers than the conventional vaccination schedule with 3 subcutaneous immunizations [20]. The enhancement of IgG and IgM titer responses in milk and serum by intramammary immunization has been explained by the possibility that cells stimulated locally in the mammary gland may

travel to local lymphatic tissues, thereby increasing systemic antibody response [49].

### **2.4. Influence of prior exposure to coliform pathogens and the effect on J5 vaccination**

It is unlikely that naturally occurring intramammary infections with coliform pathogens could confer immunological memory such as with subcutaneous *E. coli* J5 vaccination. Firstly, the variability in genotypes among wild-type coliform mastitis pathogens is very high [26, 41]. This would highly limit the formation of cross-reactive antibodies or memory cells. Secondly, plasma antibody titers were not increased after intramammary *E. coli* infections in both control and J5-vaccinated cows [21]. This suggests that the intramammary route of immunization does not result in the formation of memory cells, in contrast with subcutaneous vaccination. Similar results are, however, obtained with J5 vaccination into the supramammary lymph node and subcutaneously in the neck [49]. This suggests an important role for interaction between antigen and immune cells within lymphoid tissues in the formation of immunological memory. During naturally occurring intramammary infections with coliforms, however, the antigen will probably never reach the supramammary lymph node because the attachment of coliform bacteria to mammary epithelium, which would be a first necessary step, has never been observed [8, 25]. Thirdly, older cows – which normally underwent more intramammary infections – are more susceptible to severe coliform mastitis [55]. Age or prior exposure to pathogens also did not influence antibody response to immunization with J5 or ovalbumin [29, 56]. Together, these data suggest a quite limited role – if any – for prior natural exposure to coliform mastitis pathogens in protection against severity.

### 3. CONCLUSION

The mechanism of protection against severe coliform mastitis through J5 vaccination is presently unknown. Data in the literature support the hypothesis that it may act by inducing a state of hyper-responsiveness within the mammary gland, mediated by local memory cells. These cells could contribute to the polarization of the immune response towards a Th1 response resulting in enhanced PMN infiltration upon intramammary challenge.

### ACKNOWLEDGEMENTS

This study has been supported by the Belgian Ministry of Small Enterprises and Agriculture (grant S-6014), the Belgian "Fonds voor Wetenschappelijk Onderzoek" (FWO, grant 31504200) and the Belgian "Instituut tot aanmoediging van Innovatie door Wetenschap en Technologie" (post-doctoral research grant IWT/OZM/000445 to Hilde Dosogne).

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