

Evaluation of an interleukin-2 treatment for prevention of intramammary infections in cows after calving

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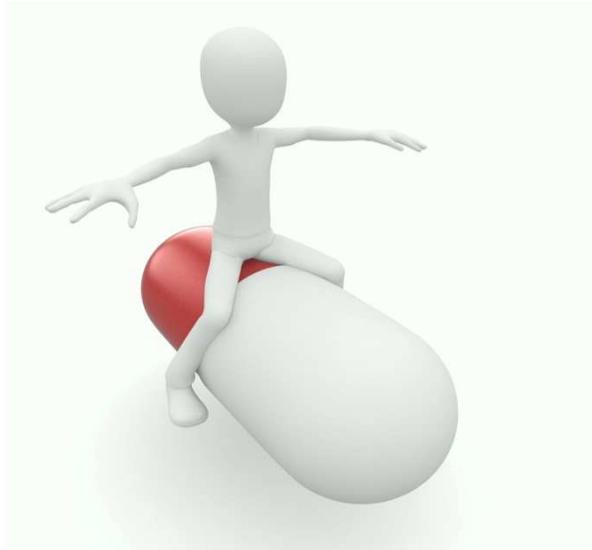


Bovine mastitis: are antibiotics totally dispensable in the veterinary practice?



- **NO !**
- Antibiotics are still a useful tool for veterinary practitioners

Is a better and reduced usage of antibiotics feasible ?



• **Yes !**

Reduced usage because of useful alternatives to antibiotics?



- In a therapeutic context probably **NO**
- **Yes** in a prophylactic context
- Reduction also feasible in terms of a more prudent, rational and effective usage

Which alternatives in prophylaxis ?

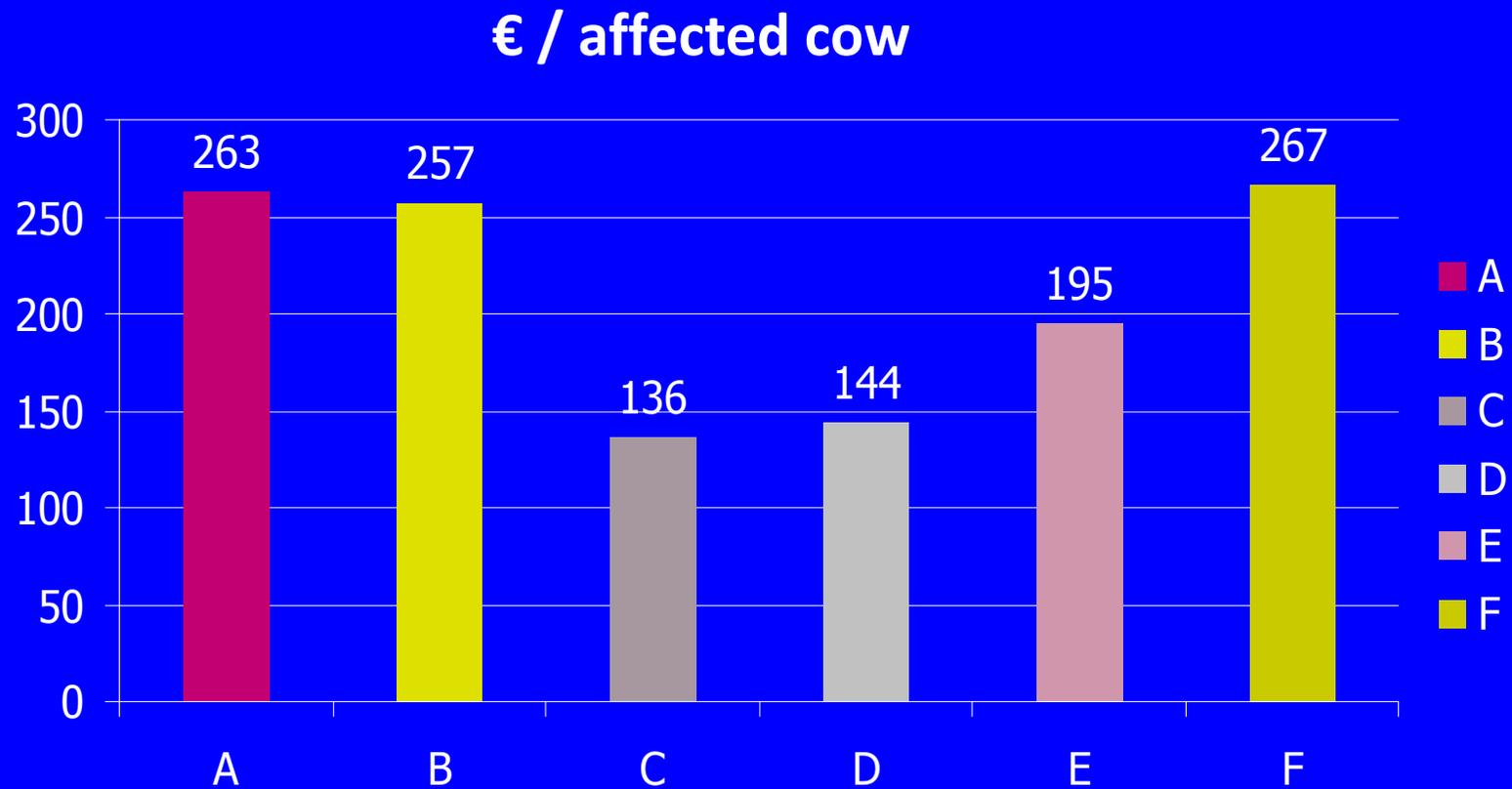


- Vaccines against mastitis: controversial efficacy under field conditions
- Scientific foundation is ill-defined (to vaccinate against common, almost ubiquitous bacterial cells ?)
- Instead, the use of Biological Response Modifiers and/or synthetic immunomodulators may be a better alternative for prophylaxis

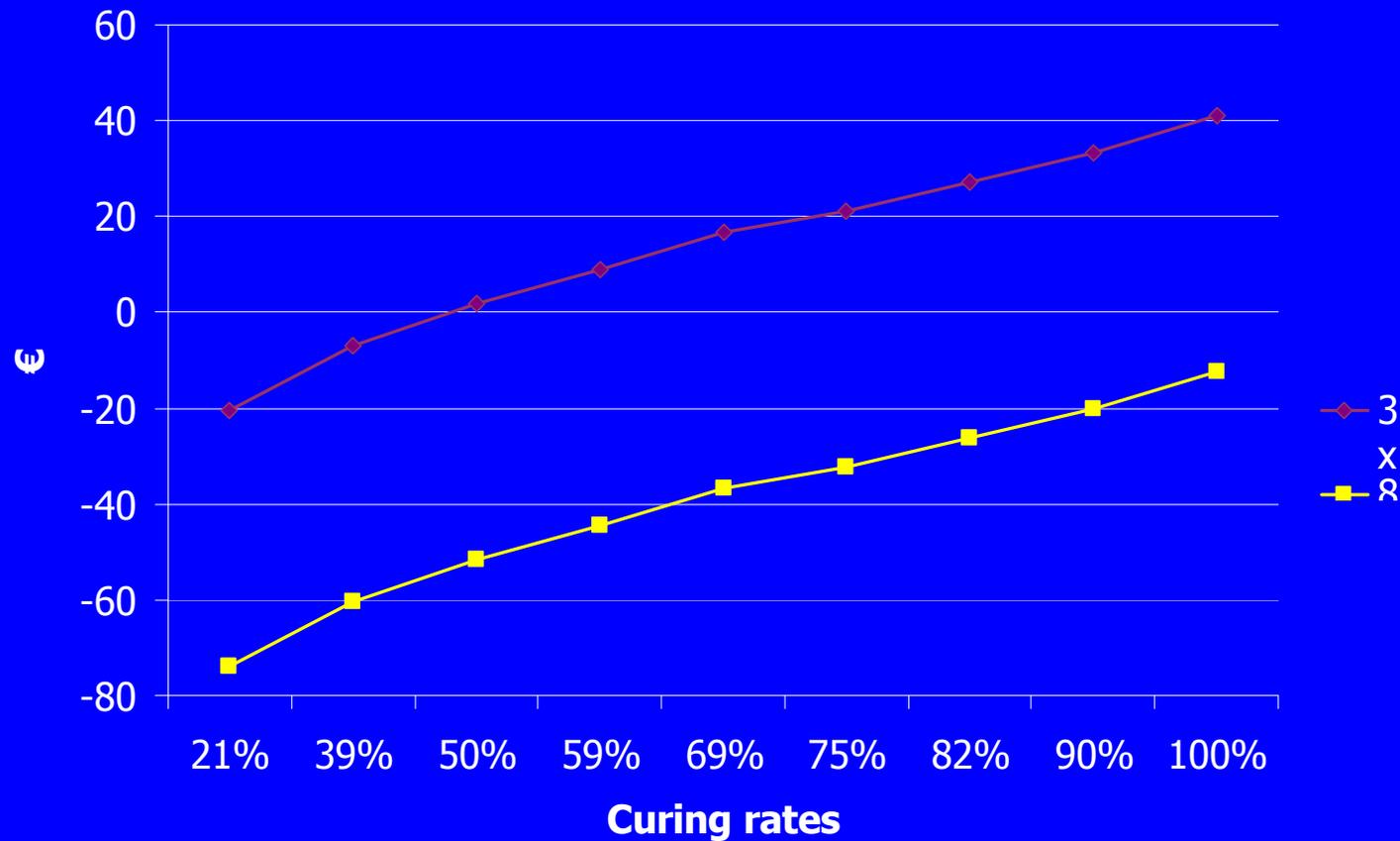
Antibiotic treatments for mastitis: disadvantages

- Therapy does not always imply healing of infected animals
- Healing of cows may not imply a financial advantage for farmers
- Therapy of subclinical mastitis aims at obtaining productive efficiency at a reasonable cost
- Is this always the case ?

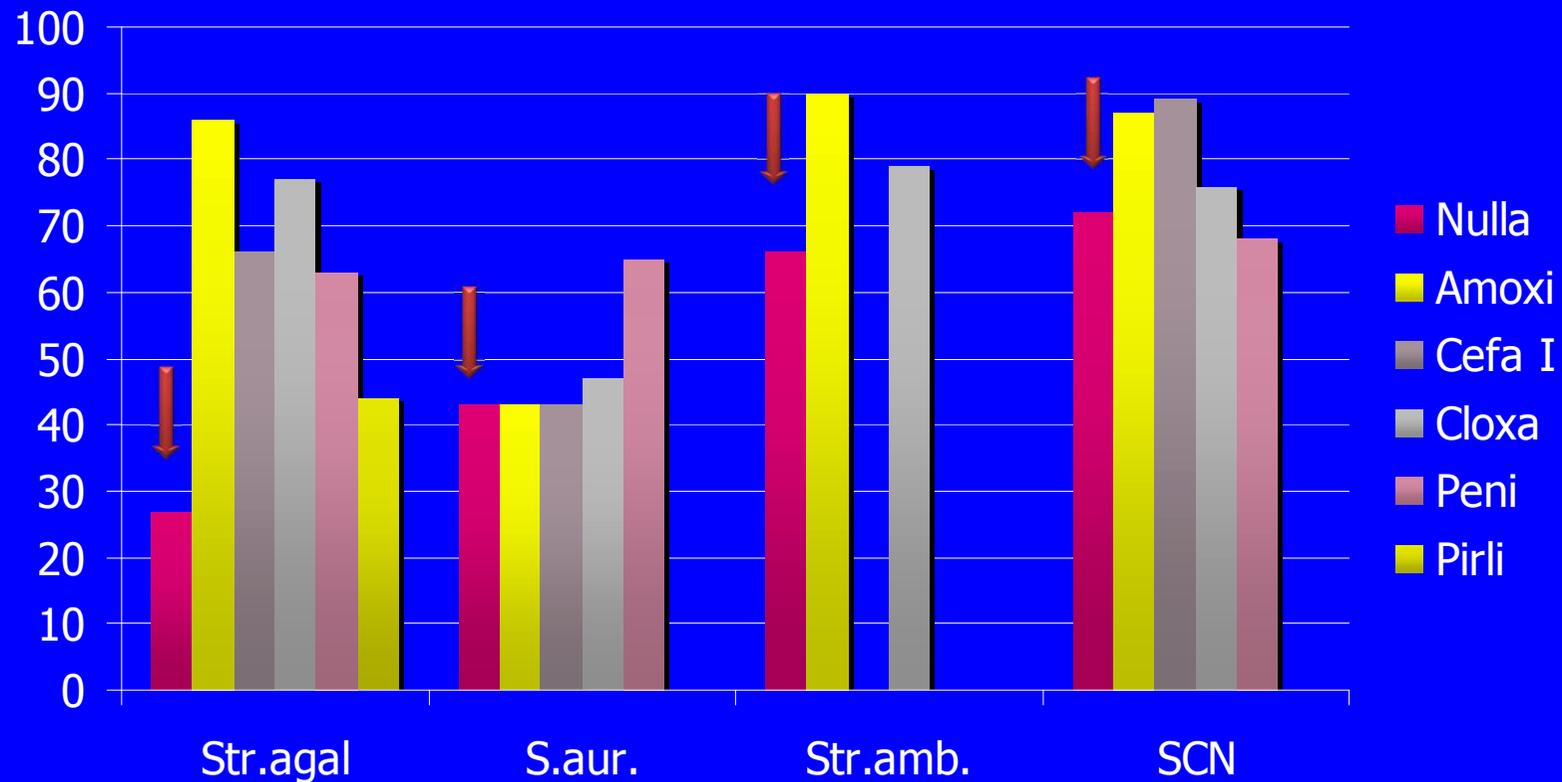
The costs of antibiotic therapies: the farm factor



Cost / benefit analysis of antibiotic treatments



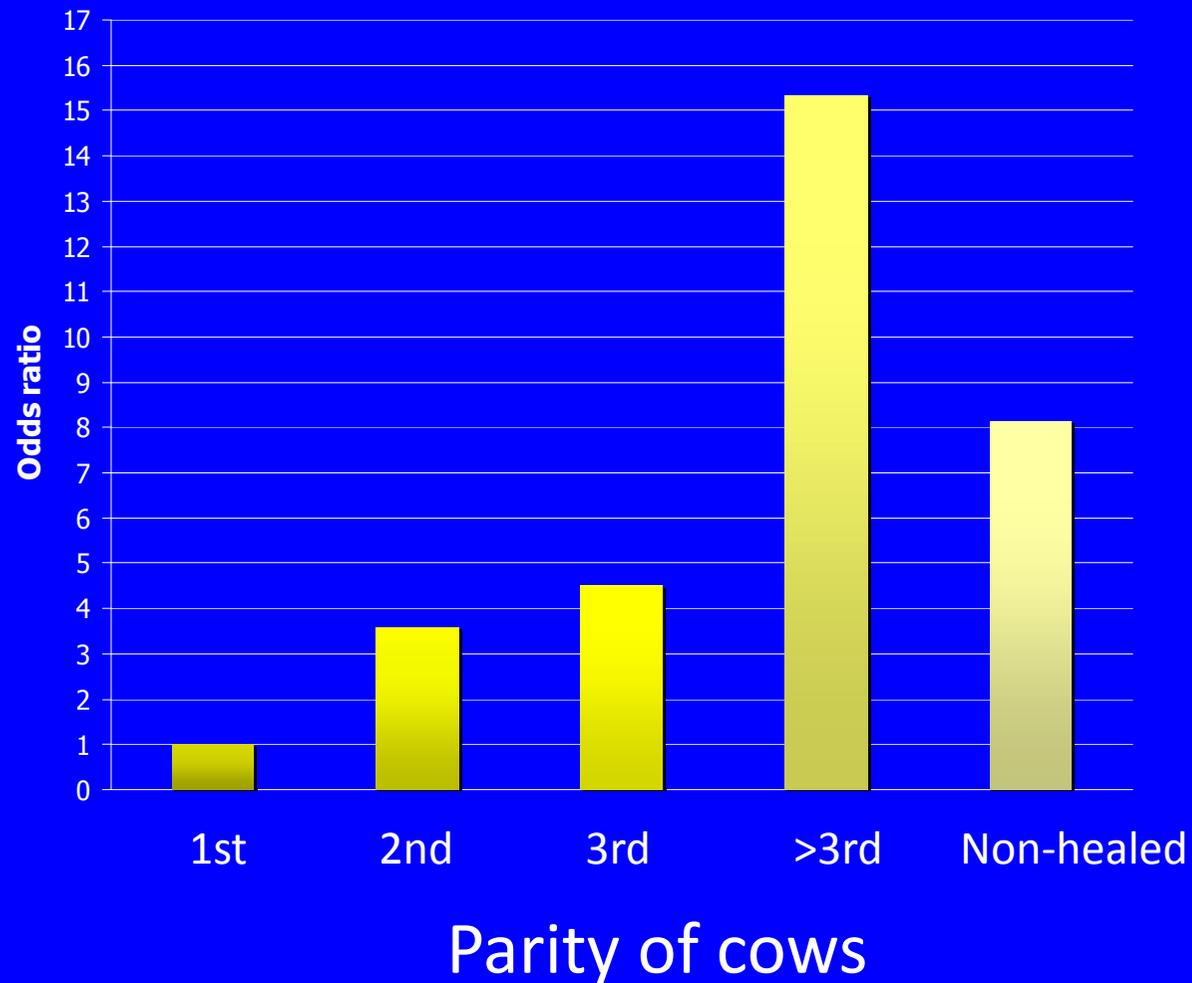
Efficacy of treatments (clinical mastitis)



Y axis: % curing rates . Red bars: no antibiotic treatment. SCN: coagulase-negative staphylococci

Wilson 1999

Probability of relapses: clinical mastitis



Take home message

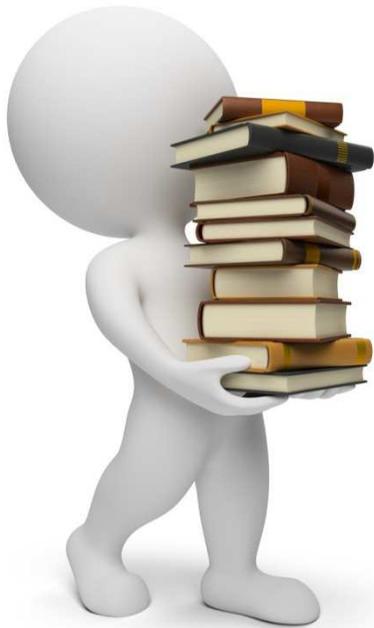


- Cases with poor prognosis should not be treated.



- **A great number of antibiotic treatments can be avoided !**

What do we need to find alternatives ?



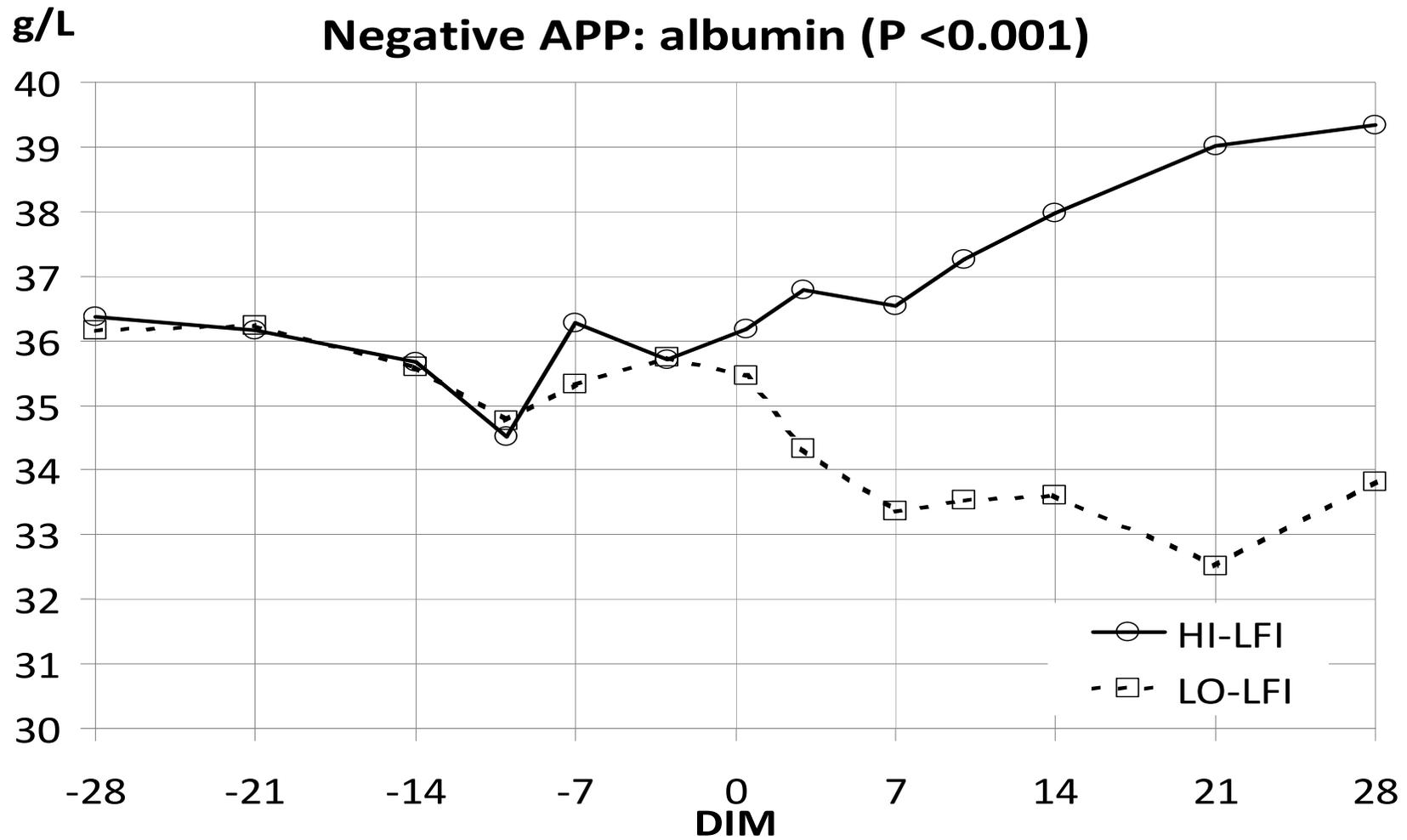
- Better understanding of mastitis pathogenesis
- The role of the immune response for udder protection
- A proof of concept focussed on alternative products which can be developed

Foundation of alternatives: an UPDATED MASTITIS MODEL



Effects of environmental and metabolic stressors

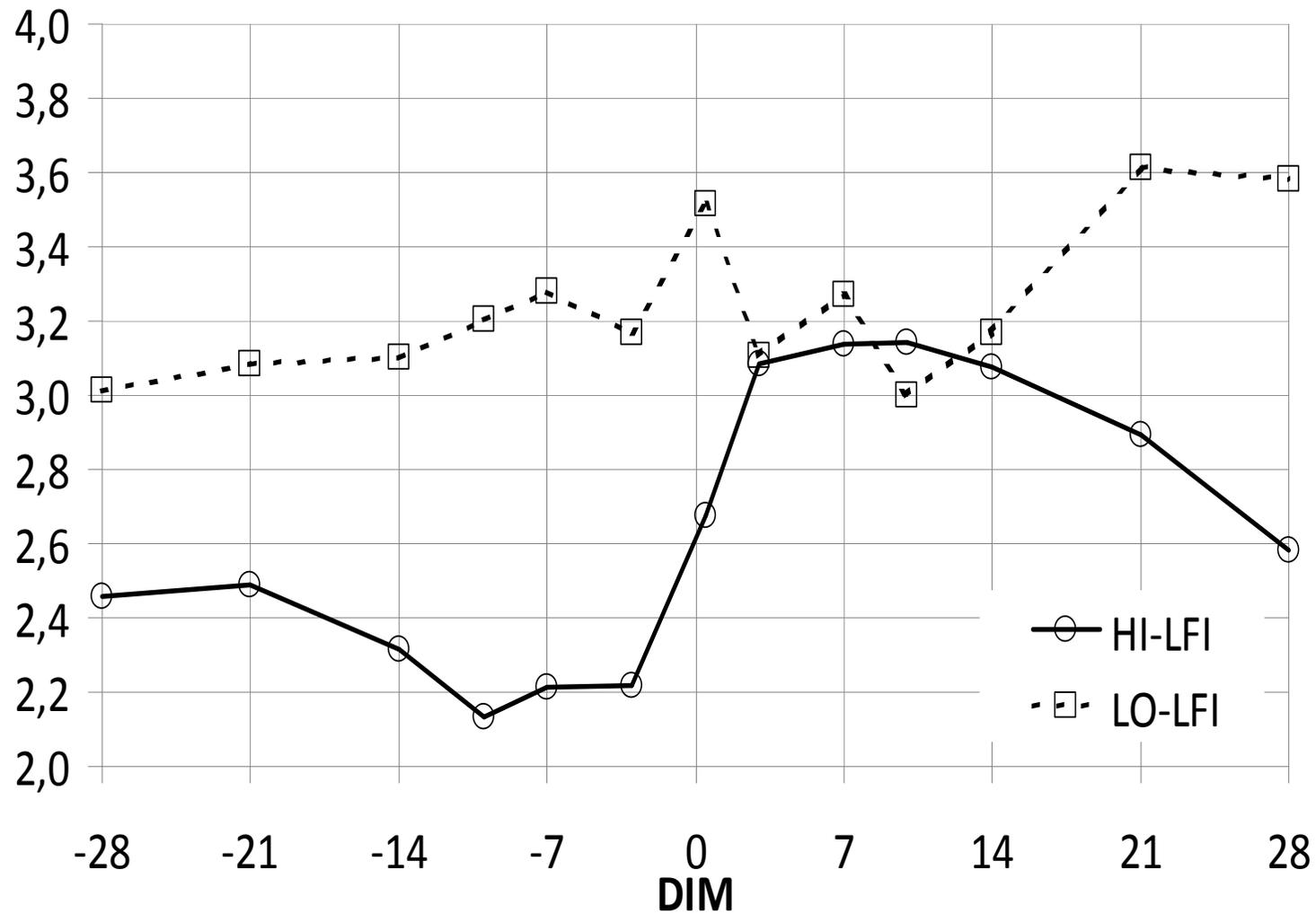
- Some cows show poor homeostatic responses to the combined effects of environmental and metabolic stressors
- Poor housing in the dry period, inadequate feeding, excessive lipomobilization, barn infectious pressure, poor cubicle quality, competition for feed and water, inadequate grouping, climatic (summer) stressors
- The early lactation period is particularly at risk for new IMI
- Some parameters of clinical immunology and clinical chemistry outline **inadequate coping strategies** with the above stressors, which is conducive to immunological deficits and production diseases like mastitis
- These parameters outline the risk for disease occurrence and antibiotic usage. **PREDICTIVE VALUE !**

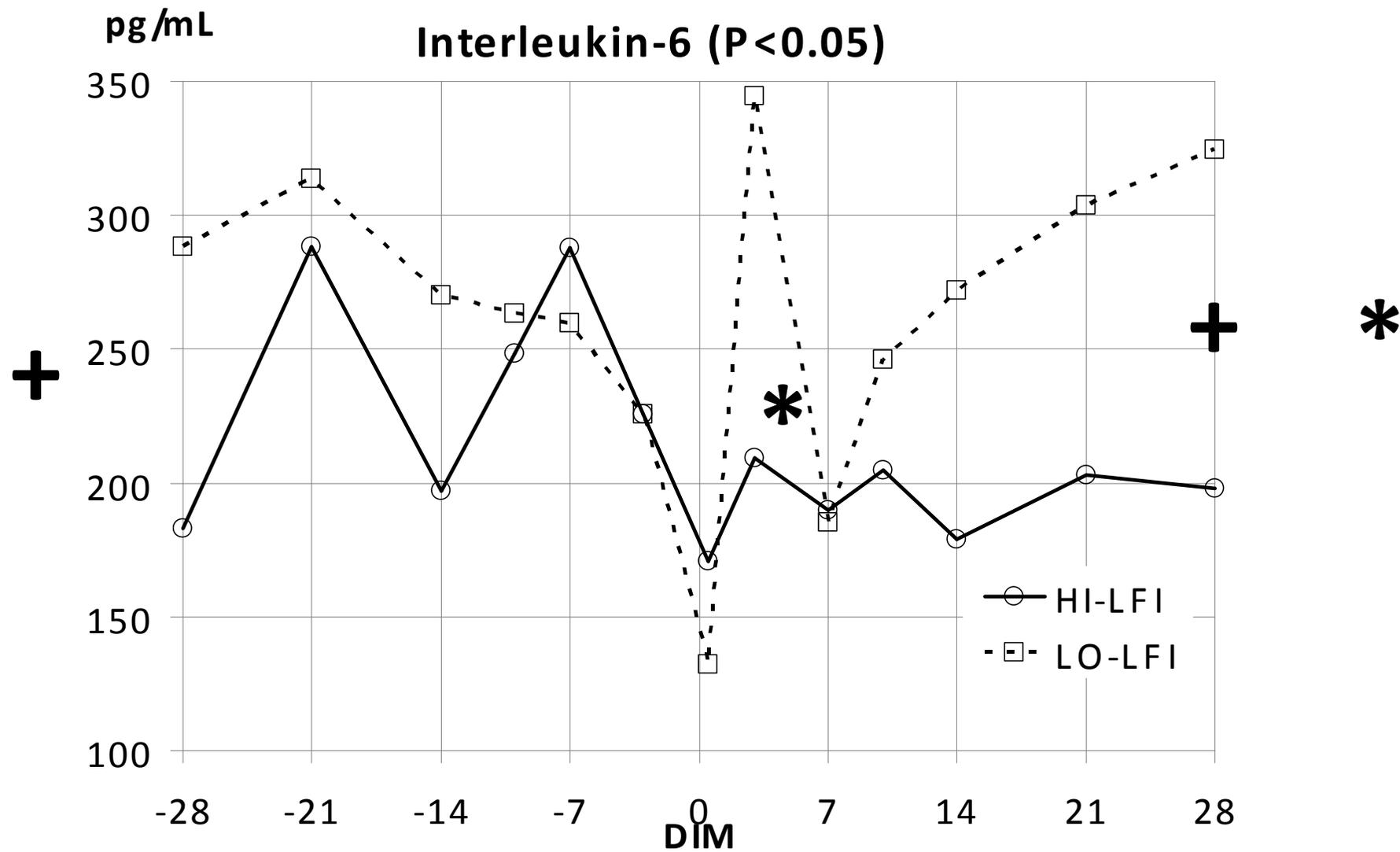


HI-LFI: healthy cows. LO-LFI: cows with a poor sanitary status after calving

APP: acute phase response

mmol/L Positive APP: Ceruloplasmin (P<0.05)

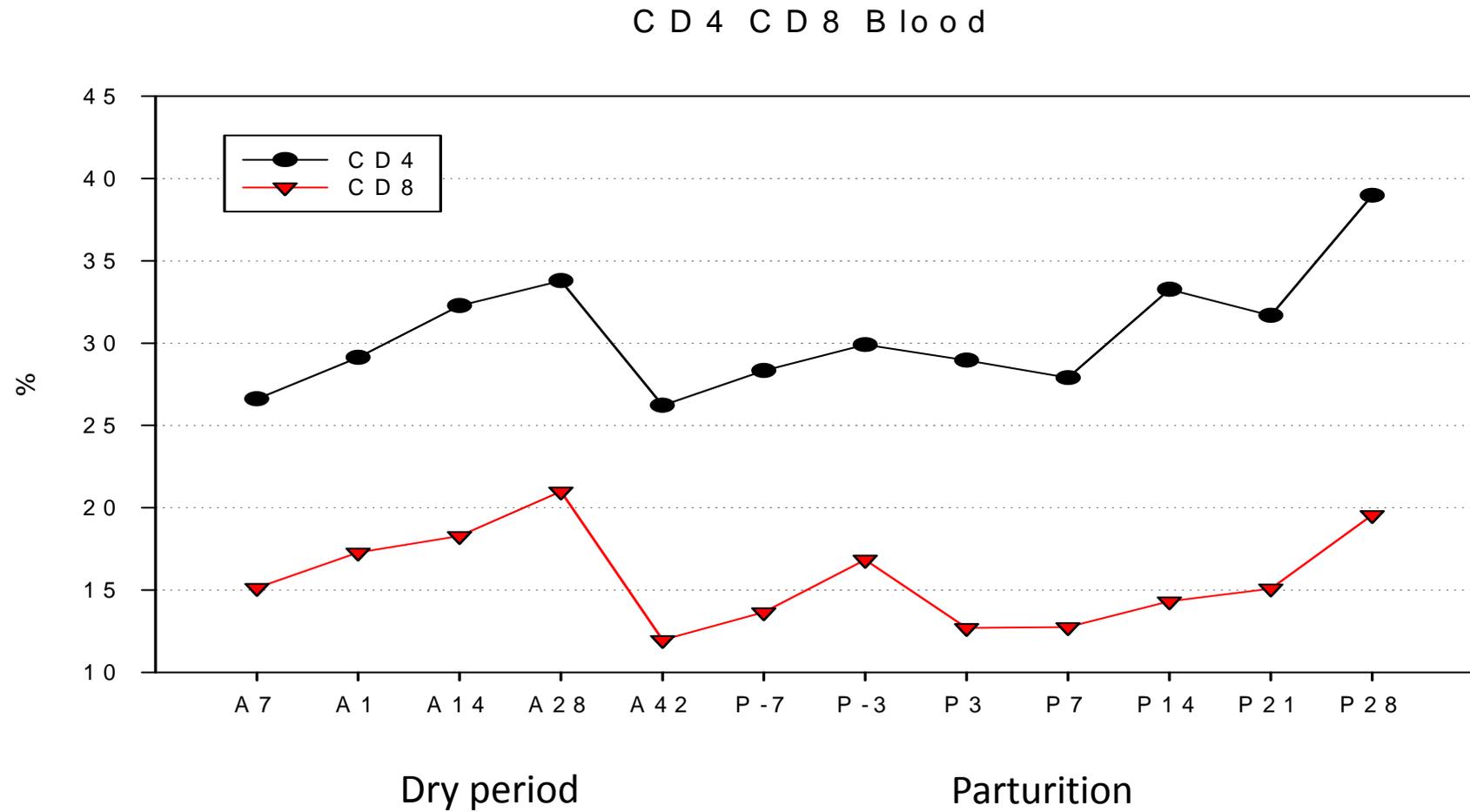




Regulation of the immune response: the MG specificity

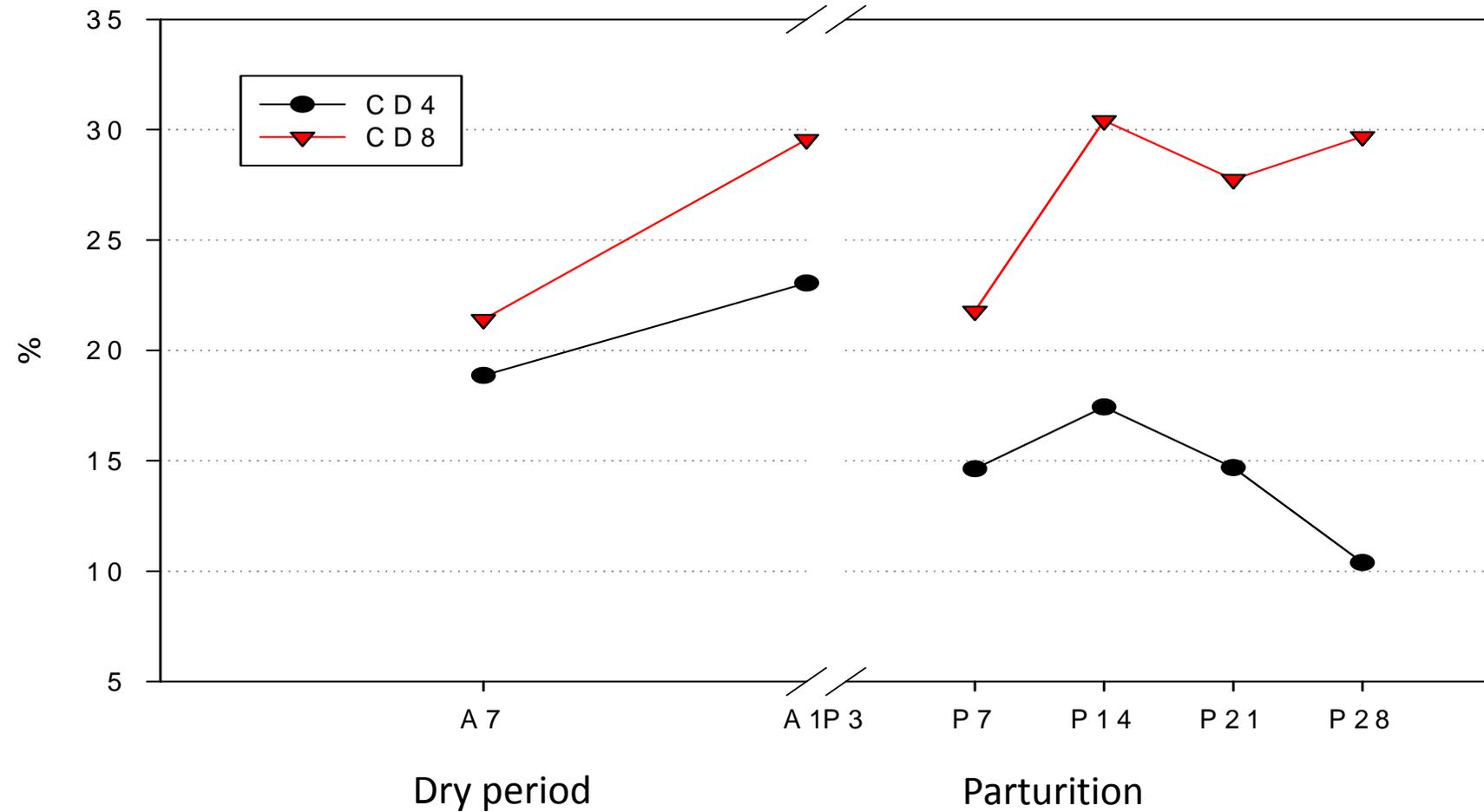
- Immune parameters in the mammary gland (MG) may be very different from those observed in blood
- Some innate immune functions in the MG show a reduced strength as compared to blood leukocytes
- Aging of PMN in the MG ?

Blood and MG immunity



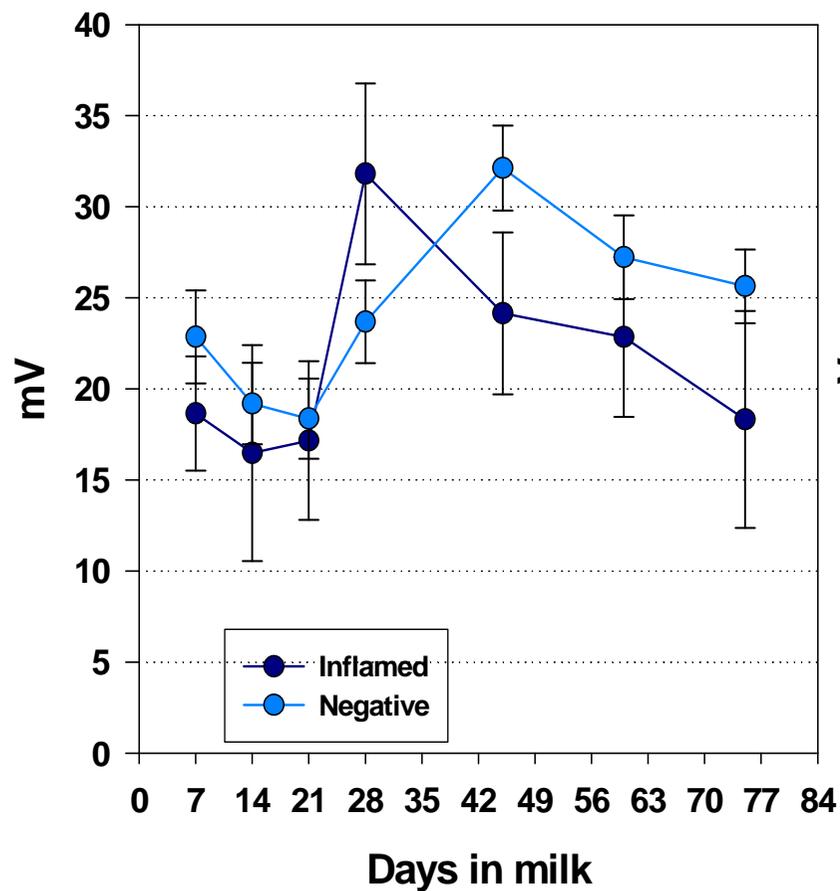
Blood and MG immunity

CD4 CD8 milk

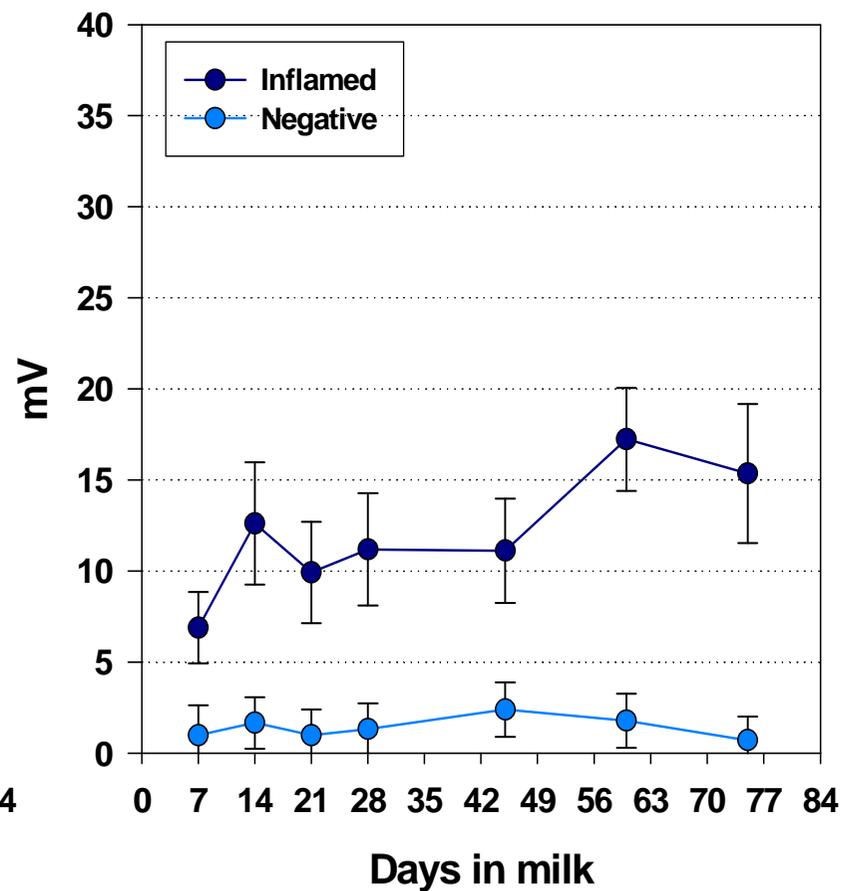


Blood and MG immunity

Oxydative burst - Blood

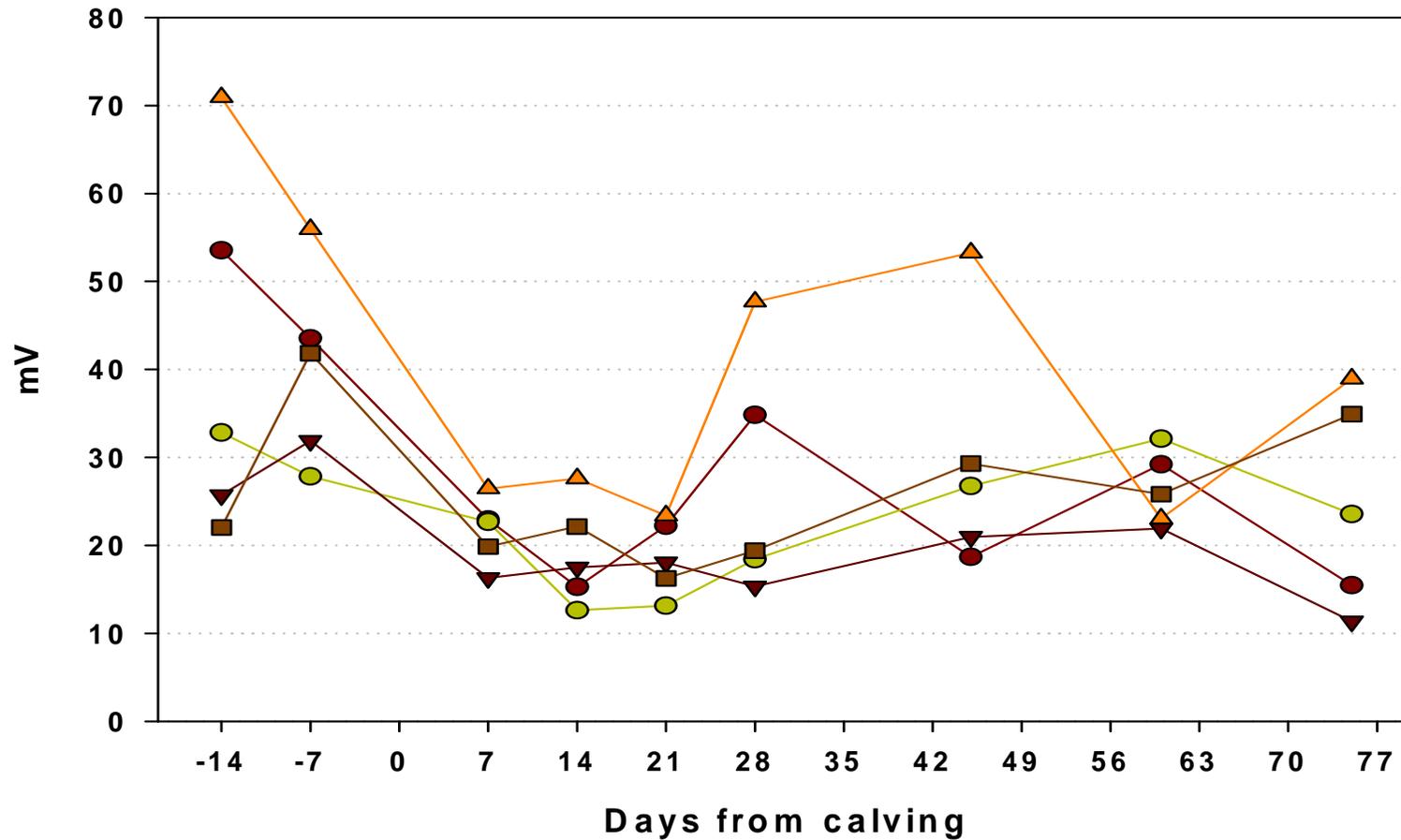


Oxydative burst - Milk

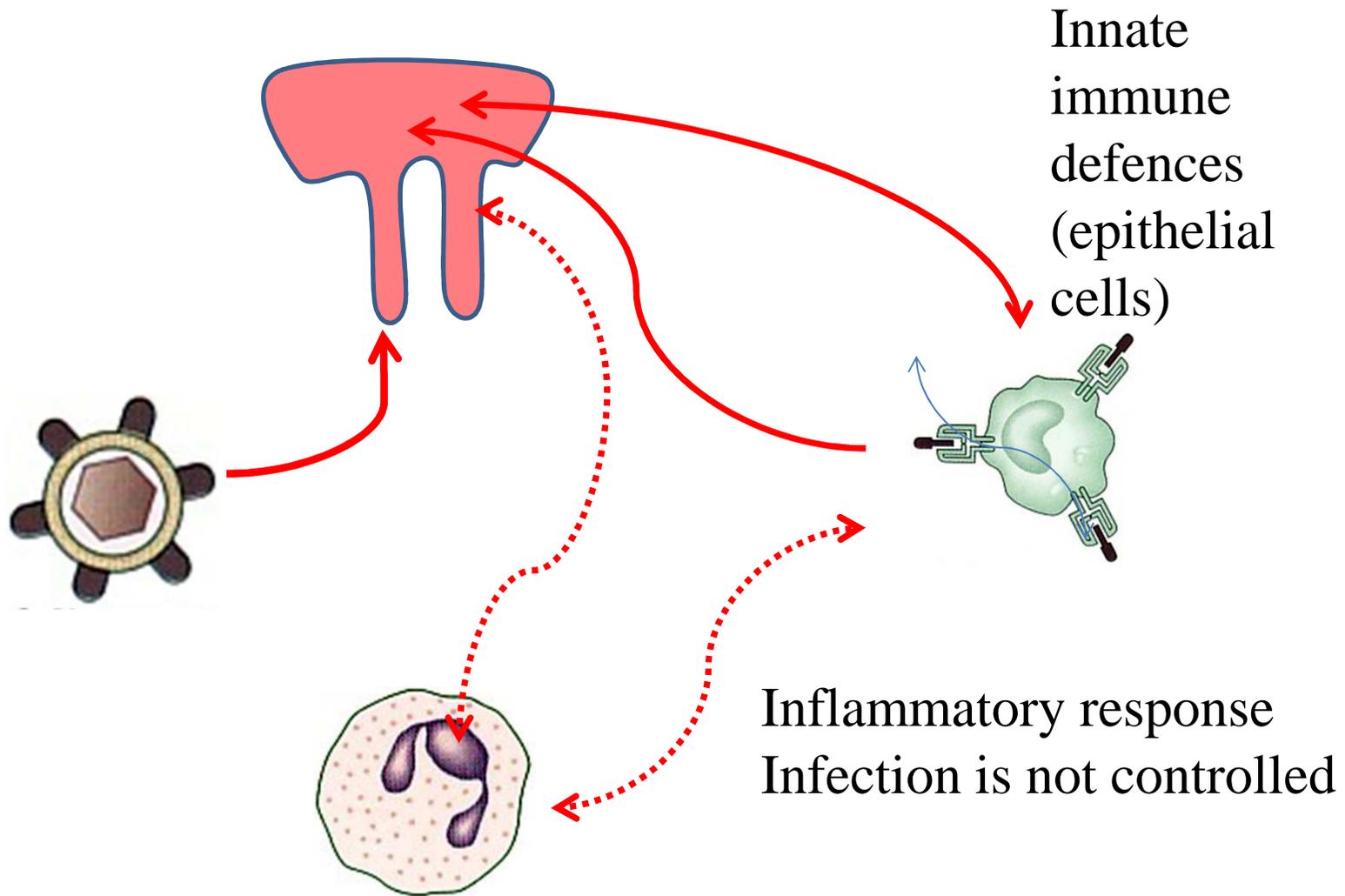


Innate immunity in MG: the farm factor

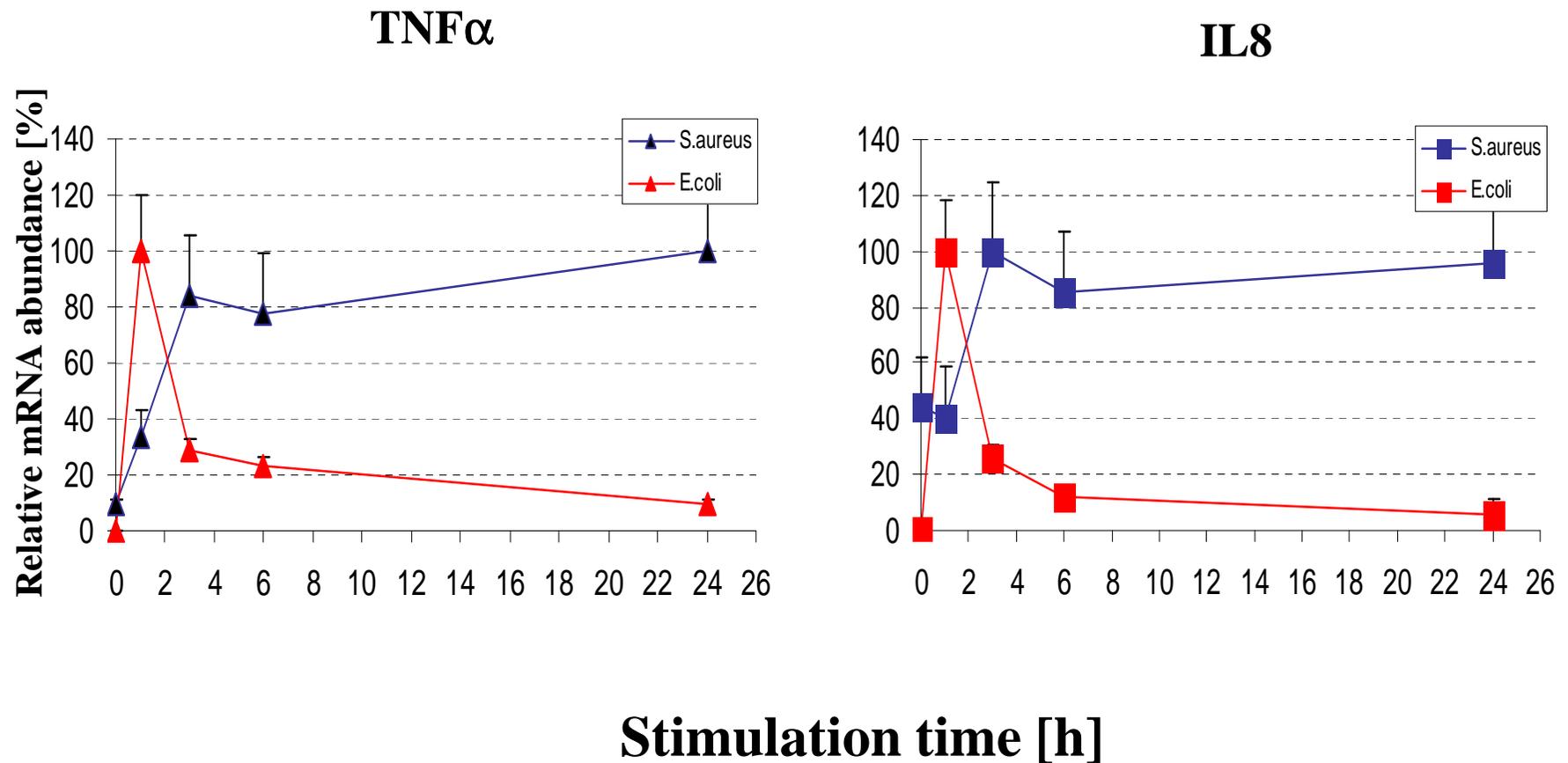
Respiratory Burst



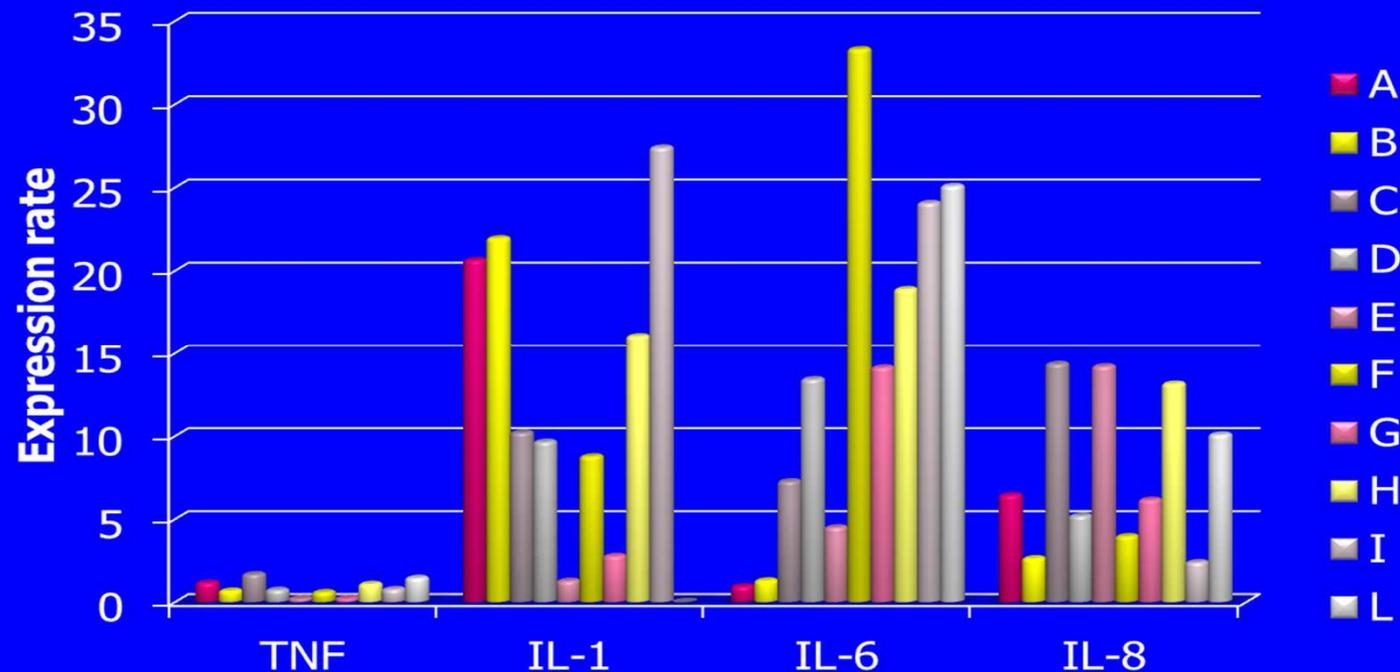
Updated model of immune response in the MG



Epithelial cells: activation of inflammatory cytokine genes



Cytokine gene expression in MG epithelial cells



A to L: 10 different *S. aureus* strains

Implications of the model



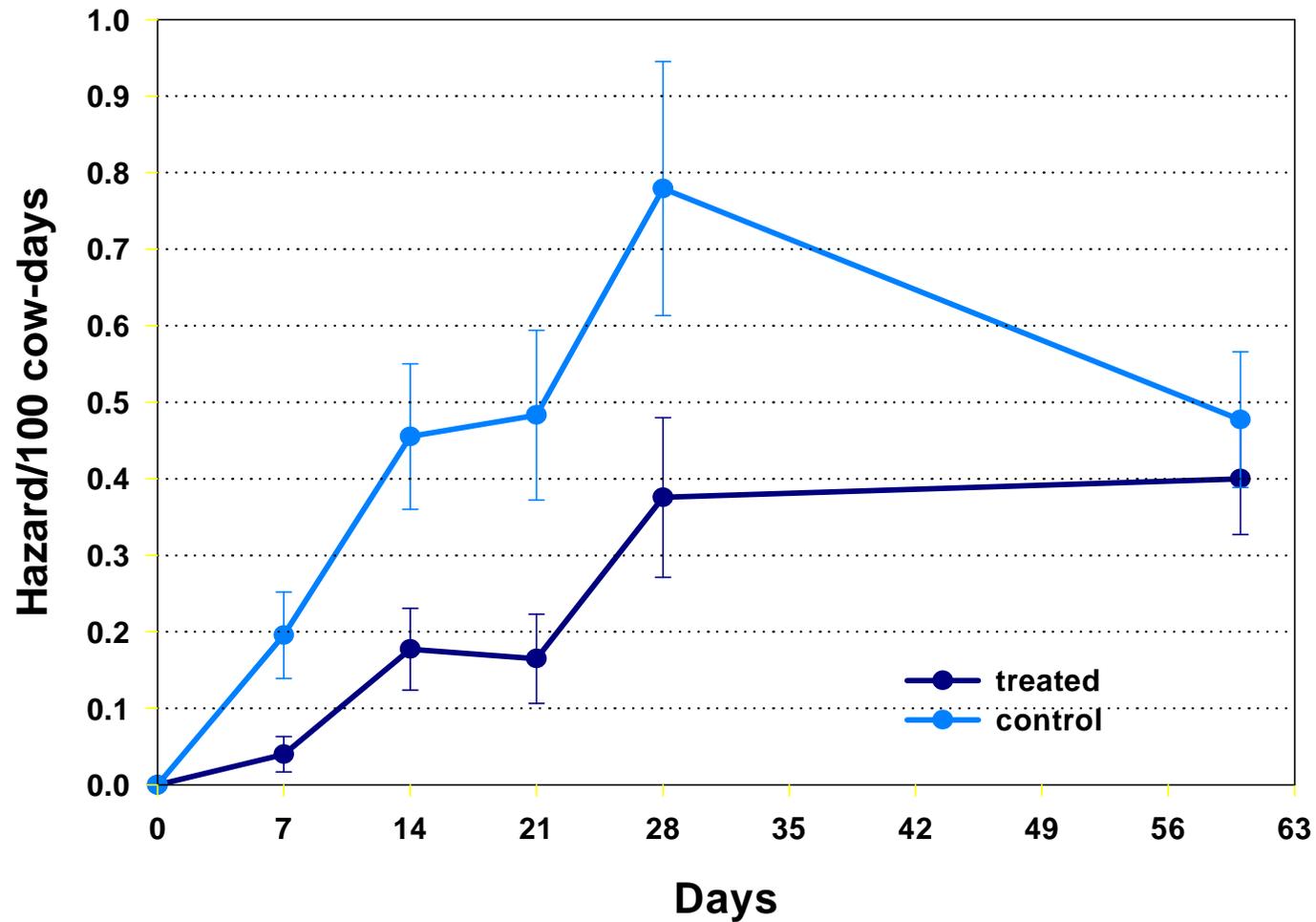
- The fundamental role of epithelial cells must be re-appraised
- Therapy and prophylaxis must sustain the protective activity of epithelial cells
- The influx of PMN into MG outlines a failure to control new IMI

How can we modulate the activity of the epithelial cells (EC)?

- EC are fully competent for innate immunity responses (receptors like TLR, NLRs, etc.)
- EC can be stimulated by cytokines / chemokines of the innate immune system
- **Two strategies**
- 1) A strategy of **active cytokine induction** in the MG (BRM or synthetic inducers)
- 2) A strategy of local / systemic **administration of cytokines**

Strategy 1

Immunomodulators - BRM



Strategy 2

General rules of cytokine actions

- Autocrine / paracrine effects are the rule
- Hormone-like, systemic effects are an exception, often related to disease occurrence
- Bell-shaped, dose/response curves (low-dose priming, high-dose suppression)
- Systemic, high-dose treatments should be avoided.

High-dose IL-2 + antibiotic treatment in the dry period: efficacy and dangers

- 1st generation Cephalosporins + 40,000,000 IU of IL-2 (Erskine R.J. et al., 1998, J Dairy Sci 81, 107-115)
- 20% more of cured quarters in the dry period after natural or experimental *S. aureus* infection
- However: increase of abortions in IL-2 treated cows (7.9 vs. 1.7%).

Efficacy of cytokine-based treatments for bovine mastitis

- High-dose, 3x, IL-2 treatment (10 mg) and rb Interleukin-1 β (200 μ g) in quarters infected with *S. aureus* (Daley et al., 1991, J Dairy Sci 74, 4413-4424).
- High-dose IL-2 treatment (3.3 or 10 mg) + antibiotics in quarters infected with *S. aureus* (Daley et al., 1992, J Dairy Sci 75, 3330-3338).
- Rb gamma-interferon and mastitis sustained by *E. coli* (Sordillo and Babiuk, 1991, Vet Microbiol, 28, 189-198)

Our project

- Use of IL-2 from a gibbon cell line (MLA 144) in serum-free medium
- Absence of endotoxin residues
- Low-dose treatment (below toxicity threshold, 3,000-8,000 Units)
- Targeted treatment in the post-calving phase (critical period for IMI occurrence)
- Parameters: new IMI, SCC , clinical cases

IL-2 Local administration

Healthy quarter frequency

Treatment	Days in milk			
	10-12	17-19	24-26	31-33
IL-2	79.5%	74.0%	72.7%	77.5%
Control	61.2%	66.7%	79.2%	74.0%
P	0.03	0.21	0.21	0.35

45 subjects. 3 herds (115-230 lactating cows). 23 treated and 22 control subjects.

Random allocation of quarters to treatment and control

1 dose (800 pg) s.c., area of the supramammary lymph node, days 3-5 after calving.

Significantly higher frequency of healthy quarters over 2 weeks after calving in IL-2 treated cows.

Zecconi et al. CIMID 2008

Immunity parameters in IL 2-treated and control cows, blood

Comparison of mean values (\pm S.D.) for blood markers in IL-2 treated and control cows

Marker (Units)	Treatment	Control
Leukocytes count (10^3 cells/ml)	7.37 \pm 0.23	7.60 \pm 0.22
Granulocytes (%)	57.62 \pm 1.20	58.16 \pm 1.18
Monocytes (%)	4.29 \pm 0.10	4.47 \pm 0.10
Lymphocytes (%)	37.47 \pm 1.14	37.75 \pm 1.12
Platelets (10^3 cells/ml)	309.13 \pm 35.37	264.67 \pm 34.60
→ Serum amyloid A (μ g/ml)	57.60 \pm 5.03	40.19 \pm 4.94 ^a
→ IgG1 (mg/ml)	4.23 \pm 4.01	10.26 \pm 3.95
→ IgG2 (mg/ml)	2.27 \pm 0.35	0.930 \pm 3.45 ^a
NAGase (units)	147.20 \pm 8.34	127.97 \pm 8.20

^a Difference between treated and control group significant at $\alpha = 0.05$.

Immunity parameters in IL 2-treated and control cows, milk

Comparison of mean values (\pm S.D.) for milk markers in IL-2 treated and control cows (all differences between groups are significant at $\alpha = 0.05$)

Marker (Units)	Treated	Control
SCC (\log_{10} cells/ml)	4.69 \pm 0.04	4.67 \pm 0.05
Milk SAA (μ g/ml)	7.76 \pm 1.07	3.22 \pm 1.32
IgG1 (μ g/ml)	19.13 \pm 1.70	26.92 \pm 2.06
IgG2 (μ g/ml)	4.80 \pm 0.50	6.77 \pm 0.61
Lactoferrin (μ g/ml)	34.60 \pm 1.14	30.64 \pm 1.13
NAGase (units)	40.66 \pm 2.06	31.58 \pm 2.49

All the differences are significant !!

SAA values in milk by health status and treatment

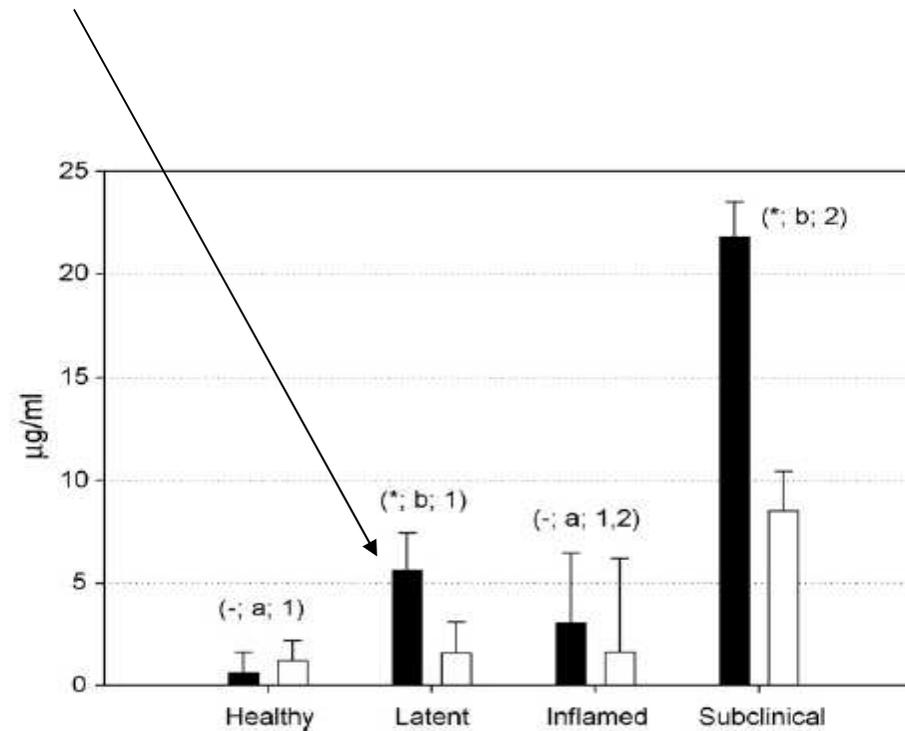
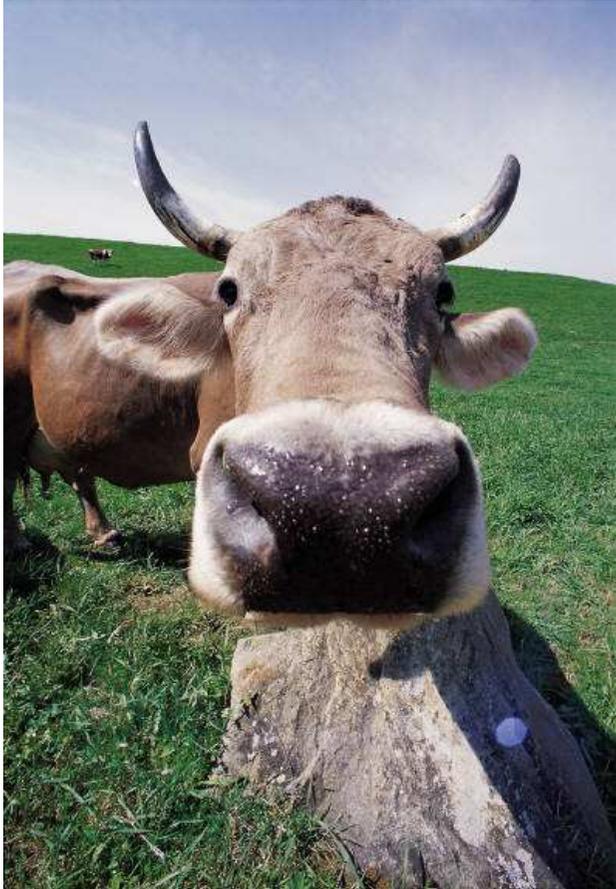


Fig. 4. Comparison of SAA mean values in milk by health status and treatment. Black bars represent IL-2 treated quarters, while open bars represent controls. The presence of an asterisk (*) means a significant difference ($P < 0.05$) between treatments within each health status class. Different letters (a, b, c) show a statistical significant difference ($P < 0.05$) among quarters by health status in the treated group. Different numbers (1, 2, 3) show a statistical significant difference ($P < 0.05$) among quarters by health status in the control group.

Take-home message

- A substantial reduction of antibiotic usage for mastitis cases is possible
- Animal welfare conditions, barn /cubicle / milking parlor hygiene, early diagnosis are of major importance
- Dispensable antibiotic treatments
- An immunostimulation approach is feasible and conducive to a better health status and food safety in the dairy sector

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**Thank you for
the attention !**