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Role of the TLR/MyD88 pathway in the modulation of the immune system activation mediated by Lactobacillus casei CRL 431

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The intestine is a complex environment where a dynamic community of microorganisms called microbiota co-exists. The microorganisms reside in the intestine in harmony with the host cells[(1)] and the establishments of these microorganisms are essential for the development of the immune system. The local effects of the microbiota on gut homeostasis[(2)] and gut immune development have been delineated[(3,4)]; however, studies have addressed the systemic effect of the microbiota[(5)] as a result of the translocation of it from the gut lumen into the circulation, but the mechanisms involved are still poorly understood[(6)]. Studies previously performed in our laboratory using conventional BALB/C mice showed that the administration of the probiotic bacterium: *Lactobacillus casei* (*L. casei*) CRL431 not only exerted activation of the innate immunity in the gut but also had effect in the systemic immunity response with enhanced of the phagocytic activity of peritoneal macrophages. Previous studies demonstrated that TLR, mainly TLR2, are involved in the intestinal signals initiated when probiotic bacteria were administrated to conventional mice in the drinking water[(7)].

In the present work we investigated the capacity of a probiotic bacterium to modulate the macrophage activation using TLR2-, TLR4- or the MyD88-deficient mice involved in the innate immunity. We also evaluated the systemic immune response by measuring the IgG anti-Ovalbumin. Mice were administered *L. casei* CRL 431 in the drinking water during 7 consecutive days or a commercial probiotic fermented milk (PFM) with the same bacteria (5 days). Afterwards, phagocytic activity of peritoneal macrophages was evaluated by *ex vivo* assays. Other groups previously administered with the probiotic bacterium or with the PFM were sensitized with three subcutaneously injections of 1% ovalbumin; the blood samples being obtained 10 days after the last injection.

According to the results obtained in the *ex vivo* assays showed that TLR2, TLR4 and the adaptor molecules MYD88 were involved in the phagocytic process of probiotics and the phagocytosis and the probiotic administration could not improve the macrophages phagocytic activity. The *in vitro* results obtained from IL-10 production by macrophages following probiotics treatment since only MyD88 is required in the production of this cytokine. The results showed that the levels of anti-Ovalbumin IgG were dependent on the TLR2 or TLR4 or MyD88 expression being dispensable for this activation.

These results showed a critical role of the TLR/MyD88 signaling pathway in some innate immune responses and in the IL-10 response induced by *L. casei*, and suggest that TLR/MyD88-independent mechanisms may participate in the immunomodulation induced by probiotics.

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