

CD3 chains play key roles in the development and activation of T cells in higher vertebrates. In the present study, a complete cDNA sequence of CD3 ϵ chain was identified from a sea bass gills transcriptome. Realtime PCR was employed to investigate the basal quantitative levels of CD3 ϵ in tissues and lymphoid organs of sea bass juveniles, with the highest expression found in the thymus followed by gut, spleen, head kidney (HK), peripheral blood leukocytes (PBL) and gills. In vitro stimulation of HK leukocytes with either T-cell mitogen PHA or sea bass recombinant IL-2, resulted in a significant increase of CD3 ϵ transcripts compared to control cultures. The CD3 ϵ cytoplasmic tail region was also identified and used to select three peptides as immunogens in rabbits, in order to produce a polyclonal antiserum. The antibody, named Ra CD3 ϵ 1, recognized the immunization peptides in ELISA and stained a band of the expected size in WB at ca. 17 kDa. The distribution and number of CD3 ϵ + lymphocyte population in the lymphoid organs, mucosal tissues and PBL were addressed in healthy fish by IHC, IIF and flow cytometry, with relatively high percentages of these cells detected among thymocytes, HK, gill and gut leukocytes and PBL, while moderate percentage were found in splenocytes. At the microscope the IIF-positive cells had the typical lymphocyte morphology and a subset of uniquely stained CD3 ϵ + IgM- cells fit the expected T cell profile. Oral stimulation with a *Vibrio anguillarum* vaccine increased the CD3 ϵ expression level in HK leukocytes, suggesting that T CD3 ϵ + lymphocytes may play important roles in the systemic protection against pathogens. Finally, the in vivo modulation of CD3 ϵ + T intestinal lymphocytes was investigated in fish fed on diets where 50% or 100% fish meal was replaced with the marine-water microalgae *Nannochloropsis* sp. biomass. IHC revealed a significant enhanced density of T CD3 ϵ + cells in the mucosa of mid intestine compared to fish fed on a control diet. These data suggest that CD3 ϵ + T lymphocytes may be involved in dietary intestinal immune responses.

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Keywords: CD3 ϵ sea bass, polyclonal antibody, immune responses, T cells

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O-085.

Identification and characterization of plasma-like cells in grass carp

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Abstract

In mammals, plasma cells are well characterized, and CD40L and IL-21 can induce plasma cell differentiation and immunoglobulin production. In this study, we developed monoclonal antibody against grass carp IgM and identified two different IgM+ B cell subsets, namely small IgM+ B cells and large IgM+ B cells. The large IgM+ B cells were further identified as plasma-like cells because they showed gene expression patterns similar with those of human plasma cells and a great capacity to secrete IgM. The small and large IgM+ B cells from either healthy grass carp or the fish stimulated with LPS or Poly (I:C) showed similar phagocytic activity. Recombinant CD40L or IL-21 alone could induce plasma-like cell generation and IgM secretion. Compared with CD40L or IL-21 alone, the combination of CD40L and IL-21 had greater effect on IgM secretion, but not on plasma-like cell generation. These results suggest that plasma-like cells in teleost fish have their own features, such as expressing cell surface IgM and possessing phagocytic activity.

Keywords: B cell, plasma-like cell, CD40L, IL-21, grass carp

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Formulation of new adjuvants to be used in fish vaccines

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Abstract

Vaccination is considered the best way of controlling infectious diseases in aquaculture. However, most of the adjuvants used in aquaculture, especially oil-based adjuvants, cause damage to fish, with consequent retardation of growth and negative effects on the welfare of the fish. In this study we developed and tested several adjuvants formulated with carboxymethylcellulose, ulvan or chitosan as the main components. We prepared and tested five formulations, including carboxymethylcellulose-methacrylate gel, ulvan-methacrylate gel, ulvan gel, ulvan-chitosan gel and chitosan gel. All formulations were tested in turbot (*Scophthalmus maximus*) and were administered alone or in combination with particulate antigen obtained from the ciliate parasite *Philasterides dicentrarchi*. Control groups of fish were injected with PBS, antigen or a vaccine containing an oil-based adjuvant (positive group). Fish were injected i.p. on days 0 and 30 with the appropriate vaccine formulation, and on day 60 the fish were examined for intraperitoneal lesions and to determine growth and specific serum immunoglobulin levels (IgM). None of the formulations caused important internal lesions (only small adhesions between internal organs and the peritoneal wall at the injection site), and they did not affect fish growth. Among all the formulations, the chitosan gel yielded the best response in terms of fish serum antibody levels. We also analyzed the immune response generated by the formulations in CD1® IGS mice. Mice were injected i.p. with FCA, aluminum hydroxide, carboxymethylcellulose-methacrylate gel, ulvan-methacrylate gel, ulvan gel, ulvan-chitosan gel or chitosan gel and *P. dicentrarchi* antigen. The best responses in terms of serum antibody levels were obtained with ulvan-methacrylate gel and chitosan gel. The values obtained were similar to those obtained in response to FCA and significantly lower than those obtained in response aluminum hydroxide. In addition, we compared the polarization of th1/th2 response in mice injected with these adjuvants. The results obtained suggest that alternative adjuvants, which induce a good immune response and do not cause important internal lesions, can be formulated for inclusion in fish vaccines.

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Keywords: Turbot, vaccine, *Philasterides dicentrarchi*, ulvan, chitosan, immune response

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Formulation of *A. salmonicida* adjuvanted vaccine for rainbow trout : Impact of the adjuvant oil origin

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