Characterization of anti-channel catfish (*Ictalurus punctatus*) T-Cell Receptor β monoclonal antibodies

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Abstract

It is well established that teleosts have the functional equivalents of T cells, which express genes homologous to mammalian T cell receptors (TCR) and to T cell accessory/signaling molecules. In catfish, the availability of cloned T cell lines offers an important model system for studying T cell function. However, due to a lack of T cell surface specific markers for most fish species, little is known about the function and regulation of teleost T cells. To this end, we have developed two anti-catfish TCR β chain monoclonal antibodies (mAb) by immunizing BALB/c mice with a TCR β constant domain recombinant protein. In Western blots, mAb 2F5-B6 recognizes a major band of ~46-50 kDa in lysates from the catfish alloantigen-dependent cytotoxic T cell line TS32.15, a size possibly representing the mature glycosylated TCR β protein. Comparatively, no reactive bands are present in lysates from the catfish clonal B cell lines, 3B11 or 1G8. Flow cytometry analyses revealed that mAb 13F11 reacts with TS32.15 T cells and not with 3B11 or 1G8 B cells. The intensity of 13F11 positive staining of TS32.15 T cells was highest on day 2 after stimulation, and then steadily declined until subsequent restimulation. This staining profile correlated with TCR message expression. Similar patterns were also observed when peripheral blood leukocytes (PBL) were isolated on a cushion of Ficoll-Hypaque and stimulated in culture with either LPS, Con A or irradiated alloantigens (3B11). Cultures were analyzed for 13F11 staining on day 4 and day 6 post-stimulation. When the cells were stained with anti-IgM mAb 1.14, no difference was observed between the various treatments. Overall, 13F11 staining levels were lower than 13F11 staining levels.

Conclusions

- Two anti-TCR β chain (IgM κ) mAbs have been developed by immunization with a TCR β constant domain recombinant protein.
- In Western blots of TS32.15 T cells, mAb 2F5-B6 reacts with a band of ~46-50 kDa. This size likely represents the mature glycosylated TCR β protein. No reactive bands were observed in B cell lysates.
- MAb 13F11 stains catfish cytotoxic TS32.15 T cells, but not B cells.
- The 13F11 staining profiles as well as TCR message expression change during the activation cycle of TS32.15 T cells. Rapidly proliferating cells appear to express less TCR β protein and message.
- ConA and alloantigen stimulated PBL showed increased 13F11 staining compared to untreated or LPS stimulated PBL.
- These data are consistent with the hypothesis that both monoclonal antibodies recognize catfish TCR β.
- Continuing efforts are underway to develop anti-catfish TCR α, β, γ and δ mAbs of the gamma isotype for more consistent reactivity.

**Does 13F11 staining correlate with TCR message expression?**

**Differential 13F11 staining of PBL stimulated in vitro with various mitogenes**

A higher percent of 13F11 staining was observed on PBL stimulated with ConA and irradiated alloantigens as compared to LPS stimulated cultures. Catfish peripheral blood leukocytes (PBL) were isolated on a cushion of Ficoll-Hypaque and stimulated in culture with either LPS, Con A or irradiated alloantigenic B cells (3B11). Cultures were analyzed for 13F11 staining on day 4 and day 6 post-stimulation. When the cells were stained with anti- IgM mAb 1.14, no difference was observed between the various treatments. Overall, 11.4 staining levels were lower than 13F11 staining levels.

**13F11 staining profiles on day 6 after stimulation**

**TCR expression analysis of TS32.15 by RT-PCR**

The geometric mean fluorescence of TS32.15 cells stained by 13F11 changes after alloantigen stimulation. Fluorescence intensity of 13F11 compared to the negative control 1.14 on days 2-8 are shown.

The specificity of mAb 13F11 using catfish 3B11 B cells and TS32.15 T cells. Cell lines were stained with 13F11 hybridoma supernatant and analyzed by flow cytometry. 13F11 reacts with TS32.15 T cells and not with 3B11 B cells.