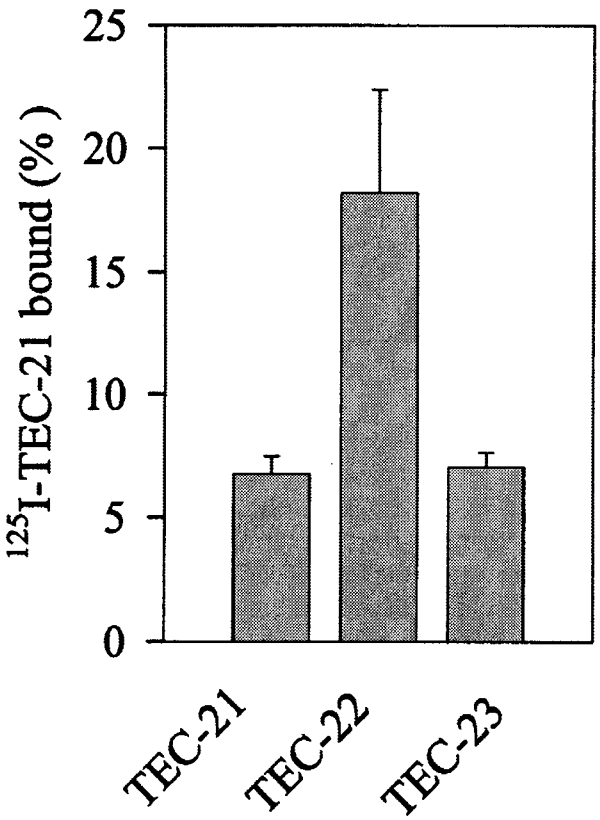

Fig. 3. Direct radioantibody-binding competition assay. RBL cells (10^6) were exposed to saturating concentrations of TEC-21, TEC-22 or TEC-23 mAbs at 37°C in complete culture medium. After 30 min the cells were washed twice in a buffered salt solution (BSS; 20 mM HEPES, pH 7.4, 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose) supplemented with BSA (1 mg/ml) followed by binding of ^{125}I- labelled TEC-21 in BSS/BSA for additional 30 min on ice. Cell-bound and unbound ^{125}I-TEC-21 was separated by centrifugation through 13% (w/v) albumin in PBS, and radioactivity was determined using a COBRA II γ counter (Global Medical Instrumentation, St. Paul, MN). The ^{125}I- TEC-21 mAb binding to control cells, untreated with antibodies, was taken as 100 %. Results are presented as means ± S.D. (n = 3).

Pretreatment of the cells with TEC-21 mAb resulted in an approximately 93% inhibition of the binding of ^{125}I-labelled TEC-21 mAb (Fig. 3). A similar inhibition was observed in cells pretreated with TEC-23. On the other hand, pretreatment with TEC-22 mAb resulted in a significantly weaker inhibition, suggesting that the two IgG_{2a} isoforms differ in their epitope specificity.

Properties

In immunoblotting assays all the three mAbs bound TEC-21 only in samples run under non-reducing conditions. It is recommended that a buffer supplemented with 1–5% bovine serum albumin (BSA) is used for blocking the non-specific binding of the mAbs to nitrocellulose, as well as for their dilution, because buffers with powdered milk reduce the binding efficiency. The antibodies differ in their pI's (TEC-21: 6.2–6.4, TEC-22: 8.0–8.2, and TEC-23: 7.0–7.2).

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