

FUNCTIONAL EXPRESSION OF THE P2Y₁₄ RECEPTOR IN MURINE SPLEEN DERIVED T-LYMPHOCYTES

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Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analysis has previously shown that the P2Y₁₄ receptor is expressed in peripheral immune cells such as lymphocytes and neutrophils (Moore *et al.*, 2003). Furthermore, although in transfected cells the P2Y₁₄ receptor couples to pertussis toxin-sensitive G_{i/o} protein(s) the functional coupling of endogenously expressed P2Y₁₄ receptors to the inhibition of adenylyl cyclase activity has not been reported (Chambers *et al.*, 2000). Therefore, the primary aim of this study was to determine whether the P2Y₁₄ receptor is functionally expressed in enriched populations of murine spleen-derived T and B-lymphocytes.

Balb/c mice were sacrificed by cervical dislocation and spleens removed under aseptic conditions. Total RNA was isolated from whole spleen and enriched populations of spleen-derived T and B-lymphocytes using RNAwizTM. cDNA synthesis was performed using random primers and M-MLV reverse transcriptase and followed by PCR analysis using P2Y₁₄ receptor specific primers: forward 5'-TTCTGGGTCGTGTTTCTTCTG-3' and reverse 5'-CGAGAGTAGCAGAGTGAATTC-3'. Measurements of [³H]cAMP accumulation were performed using cells pre-labelled with [³H]-adenine as described previously (Germack & Dickenson, 2004). T-cell proliferation was monitored by measuring [³H]-thymidine incorporation.

RT-PCR analysis detected the expression of P2Y₁₄ receptor mRNA in whole murine spleen and enriched populations of T- and B-lymphocytes. Importantly, PCR reactions using GAPDH primers performed prior to cDNA synthesis indicated that the mRNA was not contaminated with genomic DNA. In T-cells UDP-glucose (p[IC₅₀] = 6.5 ± 0.6; n=5) induced a small but significant inhibition (22 ± 4%; n=5; P < 0.05) of 5 μM forskolin-stimulated cAMP accumulation suggesting functional coupling of endogenously expressed P2Y₁₄ receptors to the inhibition of adenylyl cyclase activity. In B-cells UDP-glucose (100 μM) had no significant effect on forskolin-stimulated cAMP accumulation. Treatment of T-lymphocytes with pertussis toxin (100 ng ml⁻¹, 16 h; G_{i/o} blocker) abolished the inhibitory effects of UDP-glucose on forskolin-stimulated cAMP accumulation. Finally, UDP-glucose (100 μM) significantly reduced interleukin-2 (5 ng/ml) and anti CD3 monoclonal antibody (1 μg/ml) induced T-cell proliferation by 48 ± 13% (n=5; P < 0.05) and 45 ± 16% (n=5; P < 0.05), respectively.

In conclusion, we have shown for the first time that the P2Y₁₄ receptor is functionally expressed in murine spleen-derived T-lymphocytes. These observations suggest that UDP-glucose presumably via the P2Y₁₄ receptor may play an important role in modulating immune function.

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