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Distinct roles for tetraspanins CD9, CD63 and CD81 in the formation of multinucleated giant cells

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Summary

Members of the tetraspanin superfamily of proteins are implicated in a variety of complex cell processes including cell fusion. However, the contribution of individual tetraspanins to these processes has proved difficult to define. Here we report the use of recombinant extracellular regions of tetraspanins to investigate the role of specific members of this family in the fusion of monocytes to form multinucleated giant cells (MGC). In contrast to their positive requirement in sperm–egg fusion, previous studies using antibodies and knockout mice have indicated a negative regulatory role for tetraspanins CD9 and CD81 in this process. In an *in vitro* model of fusion using human monocytes, we have confirmed observations that antibodies to CD9 and CD81 enhance MGC formation; however, in contrast to previous investigations, we found that all members of a panel of antibodies to CD63 inhibited fusion. Moreover, recombinant proteins corresponding to the large extracellular domains (EC2s) of CD63 and CD9 inhibited MGC formation, whereas the EC2s of CD81 and CD151 had no effect. The potent inhibition of fusion and binding of labelled CD63 EC2 to monocytes under fusogenic conditions suggest a direct interaction with a membrane component required for fusion. Our findings indicate that the tetraspanins CD9, CD63 and CD81 are all involved in MGC formation, but play distinct roles.

Keywords: CD9; CD63; monocyte fusion; multinucleated giant cell; tetraspanin

Introduction

The tetraspanins are a diverse family of membrane proteins with a wide tissue distribution in multicellular organisms.[1,2] Thirty-three tetraspanin-encoding genes have been identified in humans, and roles for the proteins in cell adhesion, motility, signalling, virus susceptibility and fusion have been described.[1–3] A key characteristic of

Abbreviations: B/B/N, BSS containing 0·2% BSA and 0·1% sodium azide; BSA, bovine serum albumin; BSS, balanced salt solution; Con A, concanavalin A; EC1, small extracellular region; EC2, large extracellular region; EC_{50}, half-maximal (40%) effective concentration; FACS, fluorescence-activated cell sorter; FBGC, foreign body giant cell; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GST, glutathione *S*-transferase; HRP, horseradish peroxidase; IC_{50}, half-maximal (50%) inhibitory concentration; LPS, lipopolysaccharide; MFI, median fluorescence intensity; MGC, multinucleated giant cell; PBS, phosphate-buffered saline; RFI, relative fluorescence intensity (relative to negative control); SRB, sulforhodamine B; TEM, tetraspanin-enriched microdomains.