RESEARCH COMMUNICATION

Glycolipid modification of $\alpha 2$ interferon binding

Sequence similarity between the $\alpha 2$ interferon receptor and verotoxin (Shiga-like toxin) B-subunit

Clifford A. LINGWOOD*†‡§ and Samuel K. YIU†

*Department of Microbiology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada, and Departments of †Microbiology, ‡Clinical Biochemistry and §Biochemistry, University of Toronto, Toronto, Ontario, Canada

Previous studies have implicated the glycolipid receptor for the *Escherichia coli*-derived verotoxin, globotriaosylceramide (Gb_3 ; $\text{Gal}\alpha 1\text{-}4\text{Gal}\beta 1\text{-}4\text{Glc}$ -ceramide), in the mechanism of $\alpha 2$ interferon signal transduction. Comparison of the amino acid sequence of the human $\alpha 2$ interferon receptor with that of the B (receptor-binding)-subunit of verotoxin shows three regions of similarity which may provide a structural basis for $\alpha 2$ -interferon-receptor/ Gb_3 interaction.

INTRODUCTION

$\alpha 2$ Interferon is a cytokine involved in lymphocyte activation [1]. Although many cultured cells are able to bind $\alpha 2$ interferon specifically [2], sensitivity to growth inhibition by $\alpha 2$ interferon is restricted to a few cell types [3]. These cells are also sensitive to the *Escherichia coli*-derived verocytotoxin 1 (VT1; also referred to as Shiga-like toxin I, SLTI). This toxin is involved in the etiology of the haemolytic uraemic syndrome [4] and primarily targets endothelial cells [5] in the blood vasculature, and B lymphocytes may also be affected [6]. In fact, the cytotoxic selectivity of VT1 for cells in culture matches sensitivity to growth inhibition by $\alpha 2$ interferon. This has led us to postulate an interrelationship between $\alpha 2$ interferon and VT at the receptor level [7]. We now report on three regions of amino acid sequence similarity between the B-subunit of VT1 and the $\alpha 2$ interferon receptor which are likely to provide the molecular basis of such an interaction.

We have previously shown, using the Daudi lymphoma cell line, which is highly susceptible to VT cytotoxicity and $\alpha 2$ -interferon-induced growth inhibition, that mutant cells selected for resistance to VT are cross-resistant to $\alpha 2$ interferon [7]. The mutant cells were shown to be selectively defective in the VT-receptor glycolipid globotriaosylceramide ($\text{Gal}\alpha 1\text{-}4\text{Gal}\beta 1\text{-}4\text{Glc}$ -ceramide, Gb_3).

Cell sensitivity to growth inhibition by $\alpha 2$ interferon has been found to correlate with a biphasic Scatchard binding curve [3,7]. Sensitivity to inhibition correlates with the small number of high-affinity, but not with the large number of low-affinity, binding sites [3]. The mutant Daudi cells selected for resistance to VT were found to be defective in this high-affinity binding site as compared with wild-type cells [7]. Since $\alpha 2$ interferon itself was not found to bind to Gb_3 , we postulated that some interaction at the receptor level resulted in the generation of a high-affinity binding site for $\alpha 2$ interferon. Such a hypothesis was further supported by the finding that Daudi cells selected for resistance to growth inhibition by $\alpha 2$ interferon [3] were cross-resistant to VT and also found to be selectively deficient in the VT receptor, Gb_3 [8]. A VT-resistant double mutant was then selected from the interferon-resistant Daudi mutant. This double mutant showed an even greater deficiency in Gb_3 and was considerably more resistant to both VT and $\alpha 2$ interferon than was the parent (interferon-resistant cell line). Of the four cell lines (Daudi cell,

VT-resistant mutant, interferon-resistant mutant and double mutant), sensitivity to VT and to growth inhibition by $\alpha 2$ interferon was directly proportional to the Gb_3 content.

It was therefore our hypothesis that an interaction between the $\alpha 2$ interferon receptor and Gb_3 within the plane of the plasma membrane resulted in a conformational change in the $\alpha 2$ interferon receptor which generated a high-affinity binding site for $\alpha 2$ interferon [7]. This in turn conferred susceptibility to growth inhibition by this cytokine. Furthermore, we have found that VT is selectively cytotoxic *in vitro* to human B cells which produce IgG, as opposed to those which produce IgM [6], and this correlates with the recent finding of a selective effect of $\alpha 2$ interferon on IgG-secreting human B-cell lines [9].

The human $\alpha 2$ interferon receptor has been cloned and sequenced [10]. Structural genes for the VT family have also been sequenced [11–13]. VT is a subunit toxin comprising an A subunit (31 kDa), responsible for inhibition of protein synthesis, and up to five non-covalently associated smaller B subunits (7–10 kDa), responsible for glycolipid-receptor binding. We have shown by reconstitution of receptor-deficient cells [14] that Gb_3 is the functional receptor for the pathological effects of this toxin.

VTs comprise a family of toxins all of which bind to Gb_3 . Shiga toxin and VT1 are identical [15]. VT2 and SLTII are distinct toxins [16,17] which show 60 % similarity to VT1 in the B subunit [12]. The B subunit of VTE (the pig oedema disease toxin also referred to as SLTIIv [18]) shows 84 % similarity to SLTII [19,20] and binds primarily to Gb_4 ($\text{GalNAc}\beta 1\text{-}3\text{Gal}\alpha 1\text{-}4\text{Gal}\beta 1\text{-}4\text{Glc}$ -ceramide) in addition to Gb_3 [21].

Comparison of the amino acid sequences shows three closely spaced regions of similarity between the B-subunit of VT1 and the $\alpha 2$ interferon receptor. These findings support our previous hypothesis that the $\alpha 2$ interferon receptor can interact specifically with Gb_3 [7] and provide a surprising example of a common sequence motif maintained over an enormous evolutionary time scale.

RESULTS AND DISCUSSION

Fig. 1 shows the alignment of the VT1 B-subunit and amino acids 5–54 of the human $\alpha 2$ interferon receptor. Three major domains of similarity (A, B, C) are seen, corresponding to amino acids 8–18 (55 % identity), 30–35 (66 % identity) and 44–53

Abbreviation used: VT, verotoxin; SLT, Shiga-like toxin; Gb_3 , globotriaosylceramide.

|| To whom correspondence should be addressed.

Fig. 1. Sequence alignment of human $\alpha 2$ interferon receptor (residues 5–54 of the mature protein) and the B (receptor binding) subunit of members of the VT family

Dashed lines indicate spaces introduced to facilitate alignment. Three similar or identical domains are shaded: A, KVE----DDN; B, F--RWN; C, Q--TGM---IK. Abbreviation: IFN, interferon.

(60% identity) of the VT1 B subunit. The sequences of similarity/identity with VT1 have been boxed and shaded.

The RWN sequence in the B domain is particularly interesting, since fluorescence spectroscopy of VT in the presence and absence of Gb₃ has indicated that this tryptophan is in, or close to, the Gb₃-binding site [22]. Site-directed mutagenesis of the VT B subunit has shown that replacement of this amino acid with glutamine results in a complete loss of receptor binding, whereas a more conservative replacement with tyrosine had little effect [23]. Replacement of the preceding arginine by cysteine, following directed chemical mutagenesis of the toxin gene, also resulted in the loss of receptor-binding activity [24]. The DDN sequence in the A domain is important, since changing the DDD sequence of VT1 B subunit to DDN by site-directed mutagenesis (asparagine is found in the VTE B subunit) results in the additional binding to Gb₄ [25], in a similar manner to VTE [21], suggesting that this amino acid may be involved in discrimination of the terminal carbohydrate bound. Site-directed mutation studies with the holotoxin also showed the preceding aspartic acid residues (at positions 16 and 17 of the toxin B subunit) to be crucial for Gb₃ binding [23].

No studies have as yet been performed to implicate the C region of similarity in toxin glycolipid binding.

The $\alpha 2$ -interferon-receptor domains showing similarity to VT are found in the extracellular N-terminal domain of the protein. Some of the amino acids within the A and B domains (Fig. 1) lie within a hydrophobic cluster which is 'duplicated' further towards the C-terminus of the $\alpha 2$ interferon receptor and is also found in the external domain of the γ -interferon receptor [26,27]. γ -Interferon modification of plasma-membrane globoside exposure has been reported for endothelial cells [28].

We speculate that these VT-similar sequences enable a specific interaction between the $\alpha 2$ interferon receptor and Gb₃ (and perhaps Gb₄) to occur laterally within the plasma membrane. Although it is unlikely that the affinity of binding is similar to that of VT, which has a pentameric binding site, this interaction may result in the allosteric generation of the higher-affinity $\alpha 2$ -interferon-binding site which has been implicated in the mediation of the biological activity of this lymphokine [3]. Thus the membrane glycolipid fraction may provide an 'accessory factor' for $\alpha 2$ -interferon signal transduction.

This work is supported by MRC Program Grant PG 11123.

REFERENCES

- Pestka, S., Langer, J. A., Zoon, K. C. & Samuel, C. E. (1987) *Annu. Rev. Biochem.* **56**, 727–777
- Lau, S., Hannigan, G. E., Freedman, M. H. & Williams, B. R. G. (1986) *J. Clin. Invest.* **77**, 1632–1638
- Hannigan, G. E., Gewert, D. R. & Williams, B. R. G. (1984) *J. Biol. Chem.* **259**, 9456–9460
- Karmali, M. A. (1989) *Clin. Microbiol. Rev.* **2**, 15–38
- Obrig, T. G., Vecchio, P. J. D., Brown, J. E., Moran, T. P., Rowland, B. M., Judge, T. K. & Rothman, S. W. (1988) *Infect. Immun.* **56**, 2373–2378
- Cohen, A., Madrid-Marina, V., Estrov, Z., Freedman, M., Lingwood, C. A. & Dosch, H.-M. (1990) *Int. Immunol.* **2**, 1–8
- Cohen, A., Hannigan, G. E., Williams, B. R. G. & Lingwood, C. A. (1987) *J. Biol. Chem.* **262**, 17088–17099
- Lingwood, C. A. (1991) *Adv. Lipid Res.* **1**, 39–55
- Johnson, S. M. (1991) *J. Immunol.* **74**, 44–49
- Uzé, G., Lutfalla, G. & Gresser, I. (1990) *Cell* **60**, 225–234
- O'Brien, A. D., Newland, J. W., Miller, S. F., Holmes, R. K., Smith, H. W. & Formal, S. B. (1984) *Science* **226**, 694–696
- Jackson, M. P., Neill, R. J., O'Brien, A. D., Holmes, R. K. & Newland, J. W. (1987) *FEMS Microbiol. Lett.* **44**, 109–114
- DeGrandis, S. A., Ginsberg, J., Toone, M., Climie, S., Friesen, J. & Brunton, J. (1987) *J. Bacteriol.* **169**, 4313–4319
- Waddell, T., Cohen, A. & Lingwood, C. A. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7898–7901
- Takao, T., Tanabe, T., Hong, Y.-M., Shimonishi, Y., Kurazono, H., Yutsudo, T., Sasakawa, C., Yoshikawa, M. & Takeda, Y. (1988) *Microb. Pathog.* **5**, 357–369
- Head, S. C., Karmali, M. A., Roscoe, M. E., Petric, M., Strockbine, N. A. & Wachsmuth, I. K. (1988) *Lancet* **i**, 751
- Ito, H., Terai, A., Kurazono, H., Takeda, Y. & Nishibuchi, M. (1990) *Microb. Pathol.* **8**, 47–60
- Marques, L. R. M., Peiris, J. S. M., Cryz, S. J. & O'Brien, A. D. (1987) *FEMS Microbiol. Lett.* **44**, 33–38
- Weinstein, D., Jackson, M., Holmes, R. & O'Brien, A. (1988) *J. Bacteriol.* **170**, 4223–4230
- Gyles, C. L., De Grandis, S. A., MacKenzie, C. & Brunton, J. L. (1988) *Microb. Pathog.* **5**, 419–426
- DeGrandis, S., Law, H., Brunton, J., Gyles, C. & Lingwood, C. A. (1989) *J. Biol. Chem.* **264**, 12520–12525
- Surewicz, W. K., Surewicz, K., Mants, H. H. & Auclair, F. (1989) *Biochem. Biophys. Res. Commun.* **160**, 126–132
- Jackson, M. P., Wadolkowski, E. A., Weinstein, D. L., Holmes, R. K. & O'Brien, A. D. (1990) *J. Bacteriol.* **172**, 653–658
- Perera, L. P., Samuel, J. E., Holmes, R. K. & O'Brien, A. D. (1991) *J. Bacteriol.* **173**, 1151–1160
- Tyrell, G. J., Ramotar, K., Toye, B., Boyd, B., Lingwood, C. A. & Brunton, J. L. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 524–528
- Gaboriaud, C., Uzé, G., Lutfalla, G. & Mogensen, K. (1990) *FEBS Lett.* **269**, 1–3
- Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6934–6938
- Gillard, B. K., Jones, M. A., Turner, A. A., Lewis, D. E. & Marcus, D. M. (1990) *Arch. Biochem. Biophys.* **279**, 122–129

Received 14 January 1992/20 January 1992; accepted 22 January 1992