Betacellulin is the major ligand of epidermal growth factor receptor pathway in the exacerbation of experimental autoimmune uveoretinitis

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Introduction

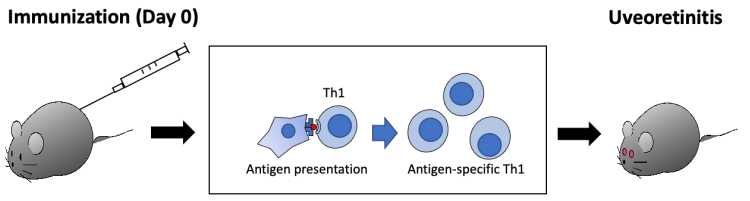
Uveoretinitis comprises various types, some of which have an acute onset, such as Behçet's disease. Epidermal growth factor receptor (EGFR) is a transmembrane protein and is a receptor for members of the epidermal growth factor (EGF) family[1)]. Epiregulin (EREG), amphiregulin (AREG), EGF, betacellulin (BTC), transforming growth factor-α (TGF-α) and heparin-binding epidermal growth factor (HB-EGF) are known to EGF family that act as ligands of EGFR[2)]. When ligands bind to EGFR, pathways such as PI3K, Ras and Jak are activated to regulate tissue growth, differentiation, and to lead wound healing and inflammation[3)].

The purpose of this study is to investigate whether the EGFR pathway is involved in the pathogenesis of acute endogenous uveitis using experimental autoimmune uveoretinitis (EAU).

Methods

【Immunization to Induce of EAU】

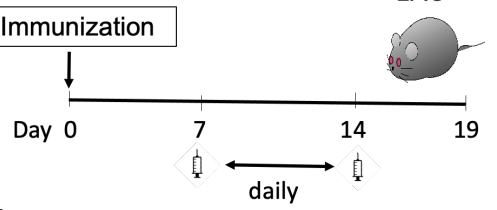
7-week-old C57BL/6 mice were immunized with human interphotoreceptor retinoid binding protein derived peptide 651-670 (hIRBPp 651-670) 200 μg emulsified with complete Freund's adjuvant (CFA) subcutaneously and were injected intraperitoneally with pertussis toxin 0.1 μg.



【Administration of EGFR inhibitor in EAU.】

Gefitinib (EGFR tyrosine kinase inhibitor) was administered intraperitoneally from day 7 to day 14. Dimethyl sulfoxide (DMSO) was administered intraperitoneally as control.

The clinical score was evaluated by two persons on a six-point scale and the pathological score was evaluated in a blinded fashion on a six-point scale in accordance with previous reports[4)].

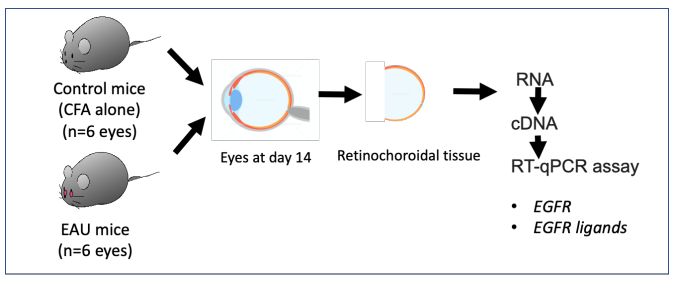


Intraperitoneal injection

Group1 : Dimethyl sulfoxide (DMSO) alone [n=6]
Group2 : Gefitinib : 3mg/day in DMSO [n=6]

【Investigation of EGFR and EGFR ligands mRNA expression in EAU】

Mice were immunized to induce EAU. As control, CFA without hIRBPp was injected. At 14 days post-immunization, eyes of the mice were removed and retinochoroidal tissue was isolated. The mRNA expression of EGFR and its ligands were quantified by PCR in the tissue.

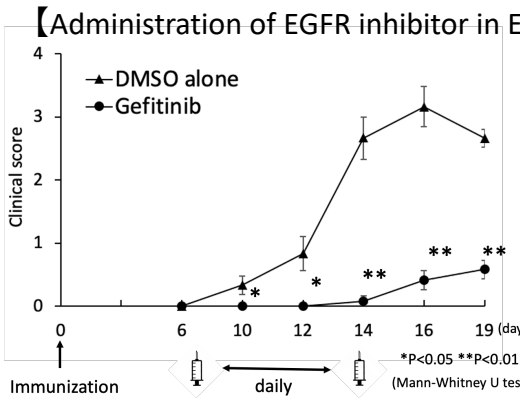


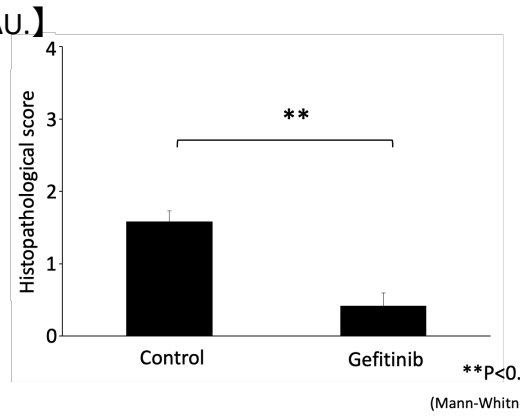
【Immunohistochemistry of BTC and EGFR in EAU】

Eyes from naïve mice and EAU mice at 21 days post-immunization were harvested, retinal sections were prepared, and immunostained for BTC and EGFR.

Results

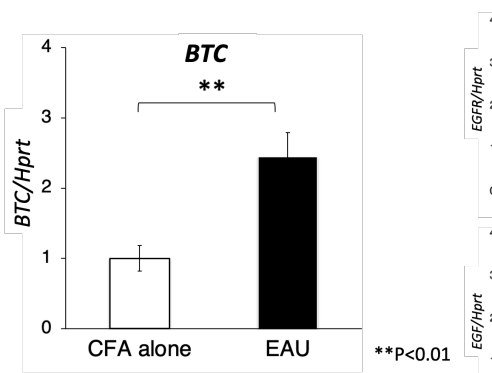
【Administration of EGFR inhibitor in EAU.】

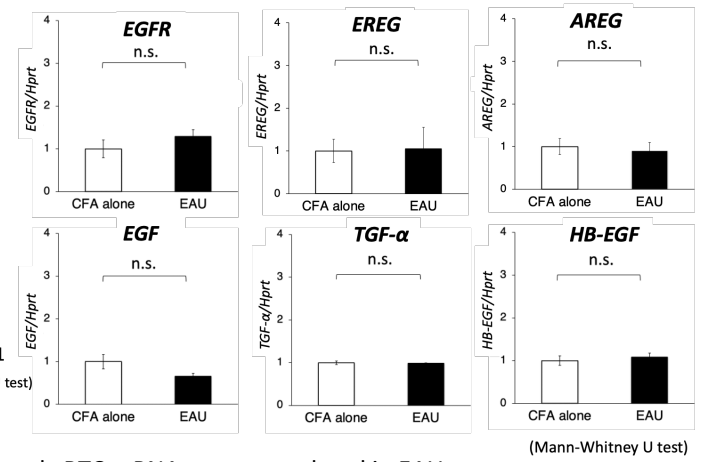




EAU was less severe clinically by gefitinib treatment after 10 days of immunization.
At day 19, EAU was milder histopathologically in gefitinib treatment group than in controls.

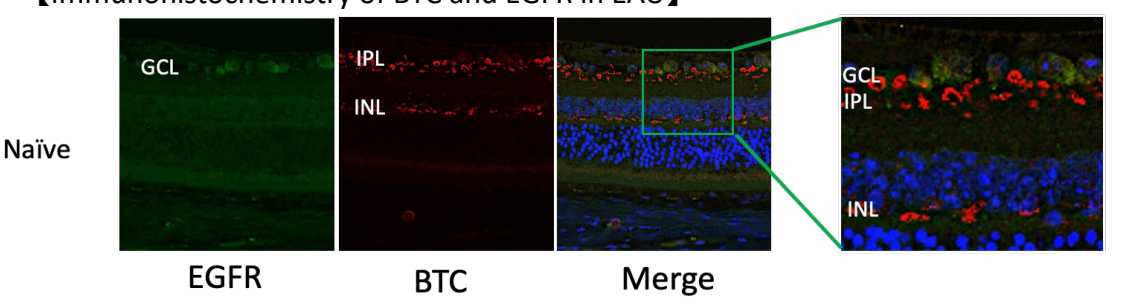
【Investigation of EGFR and EGFR ligands mRNA expression in EAU】



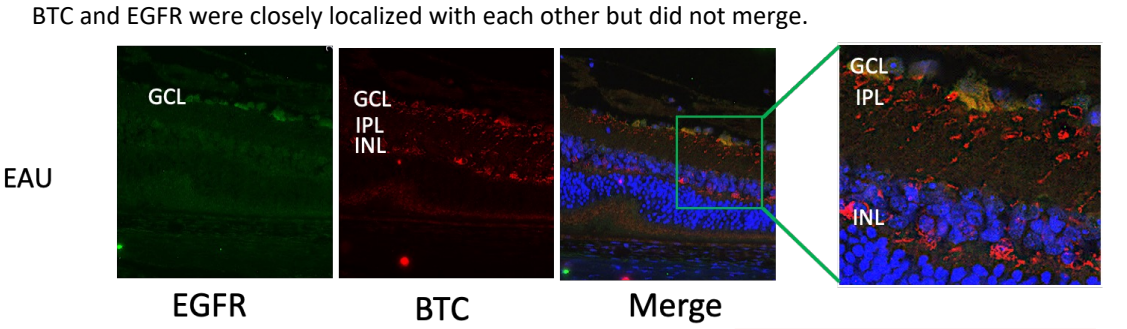


Among EGF family, only BTC mRNA was upregulated in EAU.

【Immunohistochemistry of BTC and EGFR in EAU】



EGFR was localized in GCL, which was consistent with the previous report.
BTC was localized in IPL and INL.
BTC and EGFR were closely localized with each other but did not merge.



EGFR was localized in GCL, the same as in the naïve mouse.
BTC was localized in GCL, IPL and INL.
BTC and EGFR merged in GCL.

GCL : Ganglion cell layer
IPL : Inner plexiform layer
INL : Inner nuclear layer

Summary of Results

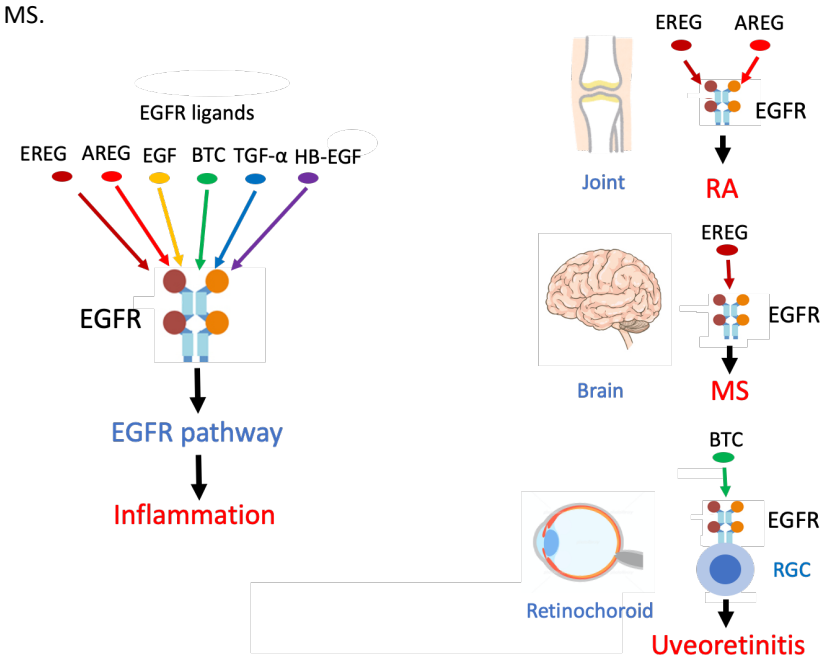
Severity of EAU was reduced by administration of gefitinib.
→EGFR pathway is involved in EAU.
BTC mRNA in retinochoroidal tissue was elevated.
→BTC was elevated in ocular tissue in EAU.
BTC and EGFR merged in GCL in EAU.
→ BTC binds to EGFR in GCL of EAU.

Discussions

It was reported that retinal ganglion cells (RGC) in GCL expressed EGFR[5)]. Therefore, BTC may bind to EGFR on RGC in GCL and exacerbate uveoretinitis.
There are some reports on BTC and its effects on the eyes. It was reported that soluble BTC was increased in the retina of diabetic mouse models, and vitreous injection of BTC increased retinal vascular permeability in mice[6)]. The increase of vascular permeability is also associated with the pathogenesis of uveoretinitis. However, there have been few reports on the role of BTC in the uveoretinitis. Indeed, this is the first report implicating BTC in the pathogenesis of uveoretinitis.

Ligand binding to EGFR is considered to exacerbate inflammation through intracellular signaling pathways. For example, it was reported that blood levels of EREG in patients with rheumatoid arthritis (RA) were significantly higher than those in healthy controls[7)]. Additionally, AREG was expressed at higher levels in the synovial tissue of RA patients compared to that of osteoarthritis controls[8)]. Furthermore, intraperitoneal administration of gefitinib significantly alleviated experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS) [7)].

The results from our studies, showing that BTC-EGFR pathway is associated with the exacerbation of intraocular inflammation, are consistent with data presented in the models of RA and MS.



Conclusions

EGFR pathway is associated with the exacerbation of intraocular inflammation, and BTC plays a particularly important role as a ligand of EGFR.

References

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