The multiple faces of tissue factor measured with laboratory assays
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Introduction

Determination of Tissue Factor (TF) in plasma is of growing interest in various pathologies including cancer, atherosclerosis, diabetes, multiple myeloma, and inflammation 1.

“Blood-borne TF” is present under 2 major forms in plasma: either as full length TF (1-263), incorporated into microparticles through its transmembrane domain, or as soluble alternatively spliced TF (asTF, 1-206) that lacks the transmembrane domain, and includes a unique N-terminal peptide 2.

There are discrepancies concerning Normal TF concentrations in plasma from one study to another, and according to the commercial assay used 3.

Materials and Methods

We developed 3 complementary assays for measuring the various TF presentations in plasma:

1. Bio-immunooassay, Zymuphen MP-TF, that permits the determination of the procoagulant activity of Microparticles exposing Tissue Factor (MP-TF).

Aim

Measurements of Microparticles exposing TF (MP-TF) and Total TF levels in normal and pathological plasmas prepared from untreated blood.

Materials and Methods

Zymutest Full length TF (FL-TF):
Capture: Mouse MoAb specific for an extracellular TF epitope, unexposed on asTF. Revelation: Polyclonal antibody (PAb) reactive with all TF forms.

Zymutest Total TF (T-TF):
Capture: Mouse MoAb specific for an extracellular TF epitope, also exposed on truncated TF.

Zymutest Full length TF specifically measures FL-TF, while Zymutest Total TF is sensitive for both forms of TF, with a reactivity of truncated compared to FL-TF of about 60%.

Results

MEASUREMENTS ON NORMAL PLASMAS

Normal “profile”
- Total TF is at about 60 pg/ml in normals.
- Full length TF and MP-TF are not detectable (<1 pg/ml).

MEASUREMENTS ON PATHOLOGICAL PLASMAS AND FOLLOWING LPS STIMULATION

Pathological plasmas
- MP-TFs are significantly increased and higher than 15 pg/ml, as TF equivalent.
- Total TF is slightly elevated or unchanged
- Full length TF is not detectable, excepted in pathological plasmas.

Conclusions

- Zymutest Full length TF specifically measures FL-TF, while Zymutest Total TF is sensitive for both forms of TF, with a reactivity of truncated compared to FL-TF of about 60%.

- Normal plasmas do not contain detectable MP-TF nor FL-TF. Mean Total TF is assayed at about 60 pg/ml (N=27; Mean±SD=62pg/ml, SD=15pg/ml), expressed as FL-TF equivalent.

- Altogether, those results suggest that in normal plasmas only the truncated form of TF is assayed, i.e. asTF, using Zymutest Total TF.

- Incubation of human blood with LPS increased MP-TF significantly, whereas Total TF and Full length TF remains unchanged.

References

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Aim: Measurement of Tissue Factor (TF) in plasma is of growing interest in various pathological states (tumor growth and metastasis, atherosclerosis, inflammation). TF can be in 3 different forms: anchored on cell membrane (monocytes, endothelial cells), in a cryptic or activated state; on microparticles’ surface (MP-TF); as an alternatively spliced form (asTF), soluble in plasma. We have developed various assays for these different TF forms: 2 ELISA assays for the measurement of full length TF:Ag (FL-TF) and “Total” TF:Ag (FL-TF and asTF); a bio-immunoassay for quantitating MP-TF activity.

Methods: FL-TF ELISA uses a capture murine MoAb specific for an extracellular TF epitope, unexposed on asTF and a second murine MoAb reactive with an extra cytoplasmic TF epitope. Tested specimen are in a diluent which enhances the assay reactivity and suppress non specific interactions (heterophilic antibodies). Total TF ELISA is a similar assay but the capture MoAb targets an extracytoplasmic epitope available on all TF forms. Assay ranges are from 0 to 500 pg/ml. This same MoAb, which does not inhibit TF activity, is used for capture in the MP-TF assay, which is revealed with Factor VIIa, Factor X, Ca++ and the Factor Xa specific substrate CS 11(65). Calibration uses relipidated TF anchored to synthetic phospholipids (0 to 5 pg/ml of TF and 0.1 nM PS/1 pg TF).

Results: In normal plasmas: FL-TF is below the detection limit (<10 pg/ml); Total TF has a mean value of 50 pg/ml. Truncated recombinant human TF (1-219) has 1% reactivity in the FL-TF (1-263) Elisa and 60% in the Total-TF. Normals were <0.2 pg/ml (TF equivalent) with the MP-TF assay but some patients were tested >5 pg/ml. Incubation of human blood with LPS increased FL-TF, asTF and MP-TF.