The multiple faces of Tissue Factor measured with laboratory assays

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Aim:
Measurement of Tissue Factor (TF) in plasma is a growing interest in various pathological states (tumor growth and metastasis, atherosclerosis, inflammation). TF can be in 3 different forms: anchored on cell membranes (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 FVIIa, Factor X, Ca++ and the Factor Xa specific substrate CS 11(65). Calibration uses relipidated TF anchored to syntetic 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/mg. Incubation of human blood with LPS increased FL-TF, asTF and MP-TF.
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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.

“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.

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

Materials and Methods

1. ZYMUTEST Full length TF (FL-TF)
   Capture: Monoclonal antibody (MCA) reactive with all TF forms.
   Revelation: Polyclonal antibody (PAb) reactive with all TF forms.

2. ZYMUTEST Total TF (T-TF)
   Capture: Monoclonal antibody (MCA) reactive with an extra cytoplasmic TF epitope, present in the N-terminal region. Tested specimen are in a diluent which enhances the assay reactivity and suppresses non specific interactions (heterophilic antibodies).
   Revelation: Polyclonal antibody (PAb) reactive with all TF forms.

3. ZYMUPHEN MP-TF
   Capture: Same as ZYMUTEST Total TF

Calibration and controls: All concentrations are expressed as FL-TF equivalent, in pg/mL. Full length recombiant TF (1-263) (AD1) has been relipidated (Zymuphen MP-TF) with synthetic liposomes (HBM) with a phospholipids/FL-TF ratio of 0,145 (10µg TF, or directly diluted, in the presence of stabilizers, (Zymutest Full length or Total TF) and lyophilized.

Plasma: Clinified normal or pathological plasmas.

LPS induction: Whole heparinized blood is incubated with Lipopolysaccharides (LPS; O111:B4 from Sigma) and plasmas are prepared by double centrifugation (15 min. at 1500g and 2 min. at 13.000g) at room temperature. Controls are the same as Zymutest Total TF.

Results

ZYMUTEST FULL LENGTH TF

- Full length TF (1-263)
- Truncated TF (1-219)

ZYMUTEST TOTAL TF

- Full length TF (1-263)
- Truncated TF (1-219)

ZYMUPHEN MP-TF

- Full length TF
- Total TF
- MP-TF

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

- MP-TF are significantly increased and higher than 15 pg/ml, 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=62 pg/ml, SD=15 pg/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