The membrane-bound but not the soluble form of human Fas ligand is responsible for its inflammatory activity

Koyo Shudo, Ryu Imamura, Shigekazu Nagata, and Takashi Suda

The ectopic expression of Fas ligand (FasL/CD95L) in tissues or tumors induces neutrophil infiltration and the destruction of the tissues or the rejection of tumors. It has been suggested that the infiltrated neutrophils are responsible for the latter phenomena. FasL is synthesized as a type II transmembrane protein, and soluble FasL is produced by a proteolytic mechanism from the membrane-bound form. We previously demonstrated that uncleavable membrane-bound FasL of mice induces IL-1 β release from inflammatory cells, and suggested that the IL-1 β enhances neutrophil infiltration. However, recent papers reported that human soluble FasL is directly chemoattractive to neutrophils *in vitro* and proposed that the soluble form of FasL is responsible for its inflammatory activity. Therefore, in this report, we investigated which form is responsible for the inflammatory activities of human FasL. We produced tumor cell lines expressing one or both forms of human FasL. Cells expressing both forms and only the membrane-bound form of FasL induced neutrophil infiltration when transplanted into the peritoneal cavity of syngeneic mice, while cells expressing only the soluble form did not. Purified soluble FasL failed to induce neutrophil infiltration *in vivo*. IL-1 β release from inflammatory peritoneal exudate and acceleration of tumor rejection were also mediated by membrane-bound but not soluble FasL. These results indicate that the membrane-bound form of FasL is primarily responsible for its inflammatory activity.

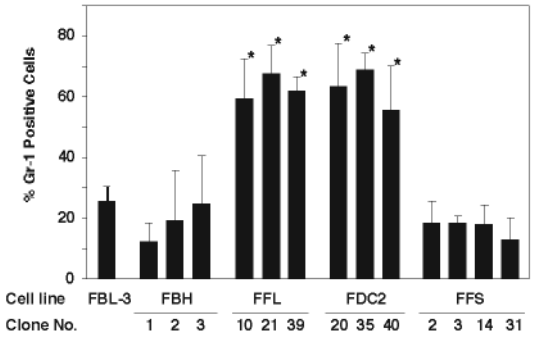

Fig. 1. Membrane-bound but not soluble FasL induces neutrophil infiltration.
FBL-3 derived transfectants expressing the membrane-bound form (FDC2), the soluble form (FFS) and both forms of FasL (FFL), and control transfectants (FBH) (4 x 10^6 cells) were injected into the peritoneal cavities of syngeneic mice. Eighteen hours later, peritoneal cells were recovered and analyzed for the proportion of Gr-1 positive cells by flow cytometry.

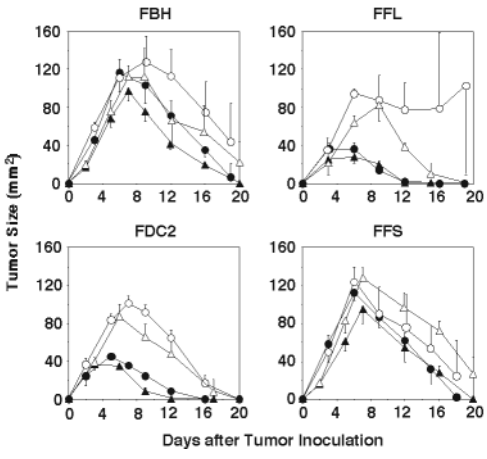

Fig. 2. Membrane-bound but not soluble FasL promotes tumor rejection.
Transfectants (2.5 x 10^6 cells) were injected into the dorsal skin of 8-12-week-old wild-type (closed symbols) or lpr/lpr female C57BL/6 mice (open symbols). Tumor size was measured at intervals of 3 days after tumor inoculation. Each point is a mean of 6 tumors in 3 mice.

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