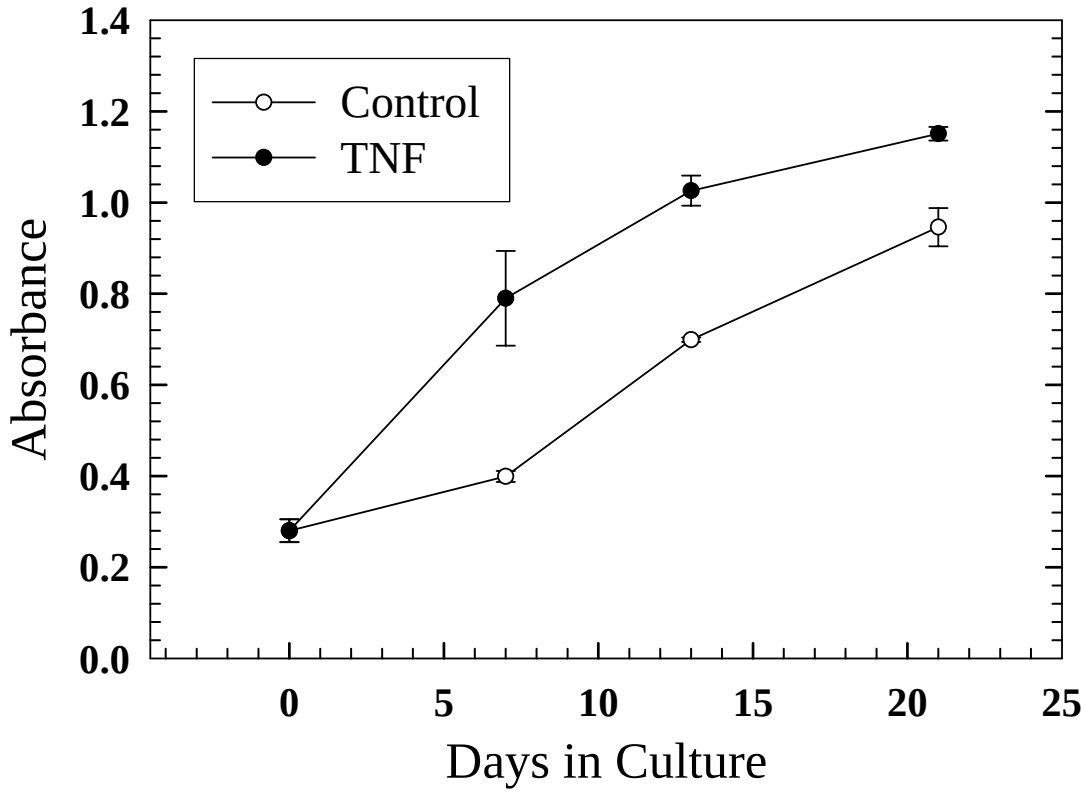
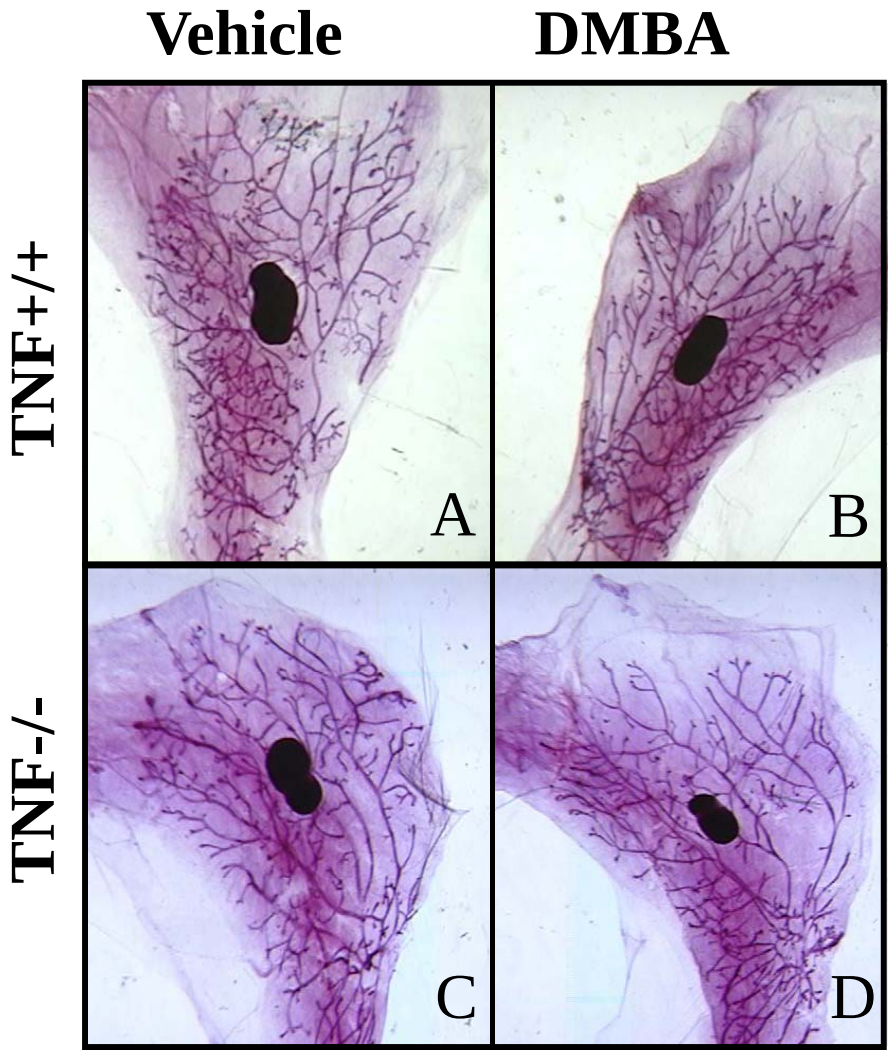
Supplementary Figure 1



Supplementary Figure 2



Supplementary Materials

Supplementary Materials and Methods

Effect of TNF status on carcinogen sensitivity of mammary glands in organ culture.

Material and Methods. One cm lengths of silastic tubing (#11-189-15G, Fisher Scientific, Pittsburg PA), sealed on the ends with Dow Corning Medical Adhesive (Factor II, Inc., Lakeside AZ) and containing 17β-estradiol, progesterone and cholesterol in a ratio of 1:1000:2002 (1), were implanted s.c. into the subscapular fat pads of 21-day old TNF+/+ and TNF-/- mice to prime the mammary gland epithelium. Fourteen or fifteen days later (1,2), the mice were sacrificed, and the #4 and 5 mammary glands removed aseptically for organ culture. To do this, the glands were carefully stretched out on siliconized lens paper (Silicone Cloth Wipes, Bausch & Lomb, Rochester, NY), placed in 100 mm dishes (2 glands per dish), and cultured in 10 ml of organ culture (OC) medium (Waymouth's medium containing 5 µg/ml insulin, 1 µg/ml aldosterone, 5 µg/ml hydrocortisone, and 5 µg/ml prolactin) at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Fresh OC medium was added one day later ("day 1"), and on day 3, 7.8 µM 7,12-dimethylbenz(a)anthracene (DMBA, Sigma) in OC medium was added for 24 hr. On day 4, the DMBA-containing medium was removed, and fresh OC medium added on days 4, 6, and 8. On day 9, the medium was changed to involution medium (IM, Waymouth's medium containing 5 µg/ml insulin and 1 µg/ml aldosterone), and the mammary glands fed every other day with IM until day 24, at which time the mammary gland whole mounts were prepared. This protocol which was adapted from that of Banerjee *et al* (3), involves the addition of a growth and differentiation medium containing insulin, prolactin, aldosterone, and hydrocortisone for the first 9 days of culture, with the carcinogen DMBA present only between days 3 and 4, and an involution medium (insulin, aldosterone) present from days 9 to 24. In Balb/c mice, it was

previously demonstrated (3) that these culture conditions permit the normal lobuloalveolar epithelium which develops during the first 9 days of culture to regress when placed in involution medium; however, preneoplastic nodule-like alveolar lesions (NLAL) arising in the DMBA-treated mammary glands did not regress, and thus could be quantified.

Supplementary Figure Legends

Supplementary Figure 1. TNF stimulates the growth of neu/erbB2-overexpressing mammary tumor organoids in primary culture. Mammary tumor organoids were isolated from NDL2-5 transgenic mice which overexpress activated neu/erbB2 in the mammary epithelium and cultured in three dimensional primary culture for up to 21 days in the absence (open circles) or presence (closed circles) of 100 ng/ml recombinant mouse TNF. Viable cell number was measured using the MTT assay and is presented as optical density at 570 nm. The TNF group is significantly different than control at the 7, 13 and 21 day time points ($P<0.05$). Each point represents the mean ± SEM of triplicate wells.

Supplementary Figure 2. Preneoplastic lesions cannot be detected in C57BL/6 mammary glands treated with DMBA in organ culture. Mammary glands 4 and 5 from each side of a TNF wild type (A,B) or TNF null (C,D) C57BL/6 mouse were treated with vehicle (A,C) or DMBA (B,D) for 24 hr starting at day 3 in culture. After allowing the glands to differentiate and then involute, they were examined for NLAL preneoplastic lesions. No differences were observed after DMBA treatment, suggesting that the mammary glands were resistant to treatment with this carcinogen. This figure, which shows the whole mounts of mammary gland 4 from the

same TNF+/+ or TNF-/- mouse, is representative of mammary glands from 7 TNF+/+ and 9 TNF-/- mice.

REFERENCES TO SUPPLEMENTAL TEXT

(1) Ginsburg E, Vonderhaar BK. Whole organ culture of the mouse mammary gland. In: Ip MM, Asch BB, editors. Methods in Mammary Gland Biology and Breast Cancer Research.New York: Kluwer Academic/Plenum Publishers; 2000. p. 147-54.

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(3) Banerjee MR, Chakraborty S, Kinder D, Manoharan K, Menon R. Cell biology of mouse mammary carcinogenesis in organ culture. In: Medina D, Kidwell W, Heppner G, Anderson E, editors. Cellular and molecular biology of mammary cancer.New York: Plenum; 1987. p. 353-79.