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Advances in Brief

Vaccinations with Tumor Cells Genetically Engineered to Produce Different Cytokines: Effectivity not Superior to a Classical Adjuvant[1]

Hanno Hock,[2] Marion Dorsch, Ulrich Kunzendorf, Klaus Überla, Zhihai Qin, Tibor Diamantstein, and Thomas Blankenstein

Institute of Immunology, Free University of Berlin, Hindenburgdamm 27, 1000 Berlin 45, Germany

Abstract

The potential of tumor cells (J558L) engineered to produce one of 5 different cytokines (interleukin 2, interleukin 4, interleukin 7, tumor necrosis factor, or γ-interferon) to give rise to systemic immunity protective against a contralateral challenge with the parental cells was analyzed. The rejection of all cytokine-producing cells appeared to induce some systemic response capable of mediating the rejection of low numbers of subsequently contralaterally injected cells, but the effect was much less obvious with higher cell numbers. The injection of any possible combination of two of the cytokine producers did not reveal any synergistic effects. The cytokine gene-transfected tumor cells were not superior to the parental cells admixed with the adjuvant *Corynebacterium parvum* with respect to their potential as immunogens to induce immunity.

Introduction

Attempts to vaccinate against cancer cells have not been rewarded with decisive success in clinical trials so far, even though considerable efforts have been taken to apply and improve various vaccination strategies to the benefit of cancer patients (1). The reasons for the apparent failure are not completely clear, especially since it was established on increasingly firm grounds that cancer cells do indeed differ antigenetically from normal cells (2). Clinical protocols have been proposed and are currently under investigation (3, 4) which use new vaccination strategies based on experiments with transplantable mouse tumors genetically engineered to produce cytokines: certain cytokines including IL-2[3] (5, 6), IL-4 (7), IL-7 (8, 9), TNF (10), and IFN-γ (11, 12) not only led to host-dependent tumor suppression after the injection of cytokine-producing tumor cells into mice, but specifically protected these mice against a subsequent challenge with parental nonproducing tumor cells. In view of the variety of cytokines which have been described to be able to positively affect immunogenicity, we addressed the question of whether some cytokines might be superior to others in this respect and whether combinations might have synergistic effects, and compared the effect of cytokine gene transfection to that of admixture of an adjuvant which has already been used both in experimental systems (13) and clinically (1).

Materials and Methods

Cell Lines. The mouse plasmacytoma cell line J558L is syngeneic in BALB/c mice and is a heavy chain loss variant of the plasmacytoma J558 (14). Culturing of J558L cells (15) and generation of all J558L sublines [J558-IL2,[4] -IL4 (16), -IL7 (17), -TNF (18), and -IFN-γ[4]] has been described; a more detailed characterization of the cell line is described elsewhere.[4] Each subline produces the respective cytokine after gene transfer. The biological activities were determined by proliferation assays with CTLL-2 (for IL2), CT.4S cells (for IL-4), and thymocytes (for IL-7); by cytotoxicity on L929 cells (for TNF); and by major histocompatibility complex class II expression on WEHI.3 cells (for IFN-γ) as described.[4] One unit was defined as the reciprocal of dilution of conditioned medium required for 50% proliferation/cytotoxicity or in ng/ml as determined for IFN-γ by a commercially available enzyme-linked immunosorbent assay (Holland Biotechnology, Leyden, the Netherlands).

Mice and Immunization Studies. Female BALB/c mice (6–8 weeks old) obtained from the Zentralinstitut für Versuchstierzucht (Hannover, Germany) were used in all experiments. Cytokine-producing or parental J558L cells (4×10^6) irradiated with 10,000 rads were injected s.c. into the necks of the mice, and the mice were challenged by s.c. injection of an indicated number of J558L cells into the belly region. For an analysis of the adjuvant's effects, J558L cells (viable or irradiated with 10,000 rads) were admixed with 100 μg of formalin-fixed *Corynebacterium parvum* (*Propionibacterium acnes*) (kindly provided by R. North, Trudeau Institute) before injection. Animals were checked for tumor growth 3 times/week for 90 days as described (17).

Results

The cell lines J558-IL-2, -IL-4, -IL-7, -TNF, and -IFN, which are all derived from parental J558L cells and produce the respective cytokine after gene transfer, were used in this study (Table 1). Due to secretion of the transfected cytokine their growth is strongly suppressed *in vivo* but not affected *in vitro* (15–18).[4] We compared the effect of prior injection of any of the cytokine producers or irradiated parental cells on the tumor growth of a subsequent contralateral challenge with J558L cells. Also, we searched for synergism by mixing two cytokine producers in all possible combinations. Fig. 1 summarizes the results of 4 independent experiments: prior injection of irradiated cells did not lead to a significant change in tumor incidence when the animals were challenged with 2×10^6 after 2 weeks (Fig. 1*d*) or 4×10^6 after 4 weeks (Fig. 1, *a* and *b*), even if a slight delay in tumor growth was observed in comparison to animals which had never encountered this tumor before (not shown). In contrast, some of the mice which had been previously injected with cytokine producers were tumor free or only transiently developed a tumor. However, there was considerable variation in the protective effect induced by each cytokine in different experiments. No cytokine producer or any combination led to the reproducible protection of more than 2 of 5 animals, regardless of whether the challenge was introduced after 2 or after 4 weeks. Although in one experiment (Fig. 1*b*) both J558-IL-7 or the combination of J558 IL-4 and TNF resulted in statistically significant protection ($P < 0.05$, Fisher's exact test) in comparison to the control with irradiated cells, this was not observed in an independent parallel experiment under the same conditions (Fig. 1*a*), nor did the effect occur when animals were challenged after 2 weeks (Fig. 1, *c* and *d*). Thus, none of the cytokine producers or their permutated combination appeared to be strikingly superior in comparison to the others regarding the capacity to generate a systemic antitumor response. Given the variation of tumor incidence, however, more subtle differences would need much larger groups in each experiment to become statistically apparent.

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[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: IL-2, -4, -7, interleukins 2, 4, and 7, respectively; TNF, tumor necrosis factor; IFN, interferon.

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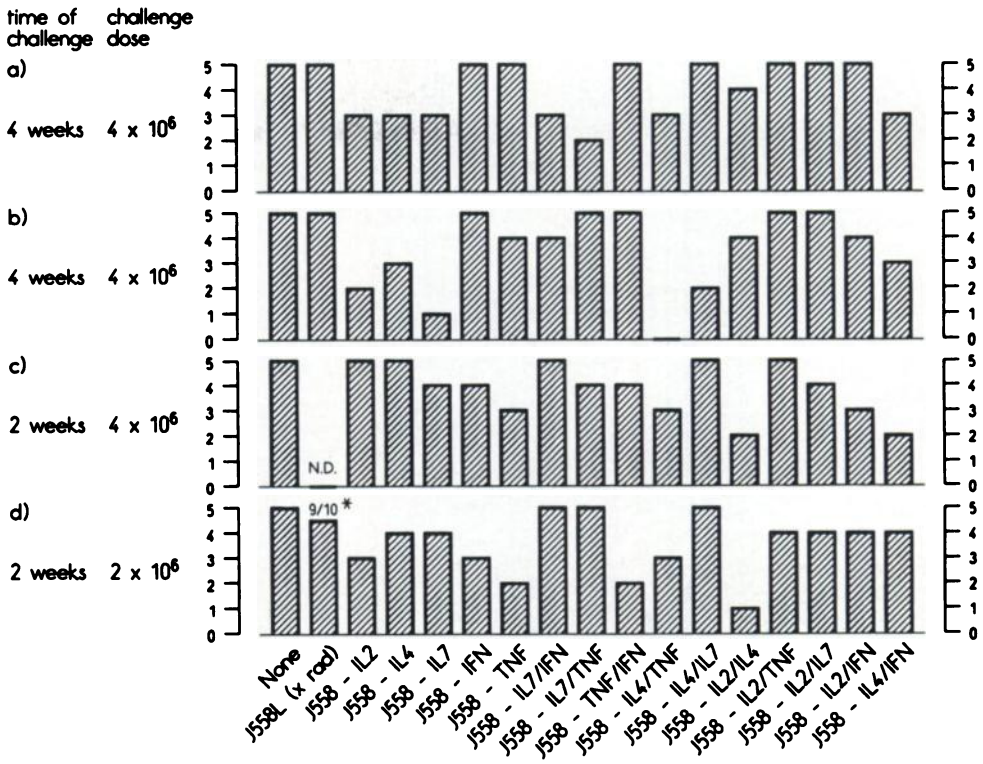

Fig. 1. Systemic protection induced by injection of single-cytokine producers or combinations of cytokine-producing cells. Mice were immunized by injection of 4 × 10^6 cytokine-producing or irradiated parental J558L cells. When combinations of cytokines were tested, 2 × 10^6 of each cytokine producer were mixed before injection. The number of mice with tumor per five mice in the experiment is given. *N.D.*, not done; *, 10 mice were challenged.

Table 1 *Cytokine-producing J558L sublines*

Cell line	Vector	Cytokine production	References
J558-IL2	pLTR-IL2	40 units/ml	footnote 4
J558-IL4	pXEP-IL4	75 units/ml	16
J558-IL7	pBA-IL7	50 units/ml	17
J558-TNF	pBA-TNF	17 units/ml	18
J558-IFN	pLTR-IFN	120 ng/ml	footnote 4

In view of the rather moderate systemic effects obtained by prior injection of cytokine producers, a comparison with the well-established adjuvant *C. parvum* (13) was performed. The growth of up to 10^6 parental J558L cells when administered locally with an admixture of 100 μg *C. parvum* was completely inhibited, but when higher numbers of tumor cells were administered the effect of *C. parvum* became incomplete (Fig. 2*a*). When mice which had been given injections of either irradiated J558L cells or of the same number of irradiated cells mixed with *C. parvum* were contralaterally challenged 18 days later with 2 × 10^6 J558L cells, no effect could be observed (Fig. 2*b*). However, when mice which had survived the injection of 4 × 10^6 viable J558L cells mixed with *C. parvum* were challenged with 2 × 10^6 J558L cells 4 of 5 mice remained tumor free (Fig. 2*b*).

Finally, we wanted to directly compare the protective effects induced by *C. parvum* admixture and the cytokine-producing cells. Therefore, mice were injected with 4 × 10^6 cytokine-producing or parental J558L cells admixed with *C. parvum*. In order to be able to more sensitively assess differences in the strength of the systemic response, groups of mice were challenged with escalating cell doses. Fig. 3 shows that most mice develop a tumor after prior injection of the cytokine-producing cells, when challenged with >1 × 10^6 tumor cells, whereas mice challenged with smaller cell numbers were obviously better protected. In contrast, prior injection of J558L cells mixed with *C. parvum* induced significantly better protection than irradiated cells ($P < 0.05$, Fisher's exact test), even when animals were challenged with 2 × 10^6 or 4 × 10^6 J558L cells. The statistical analysis of contigency tables using the χ^2 test revealed that the potencies of immunization with different cytokine producers did not differ from each other when animals challenged with all different doses were included in the analysis. However, the mixture of tumor cells and *C. parvum* was significantly superior ($P < 0.01$) to cytokine-producing tumor cells with respect to the generation of a systemic protective immune response.

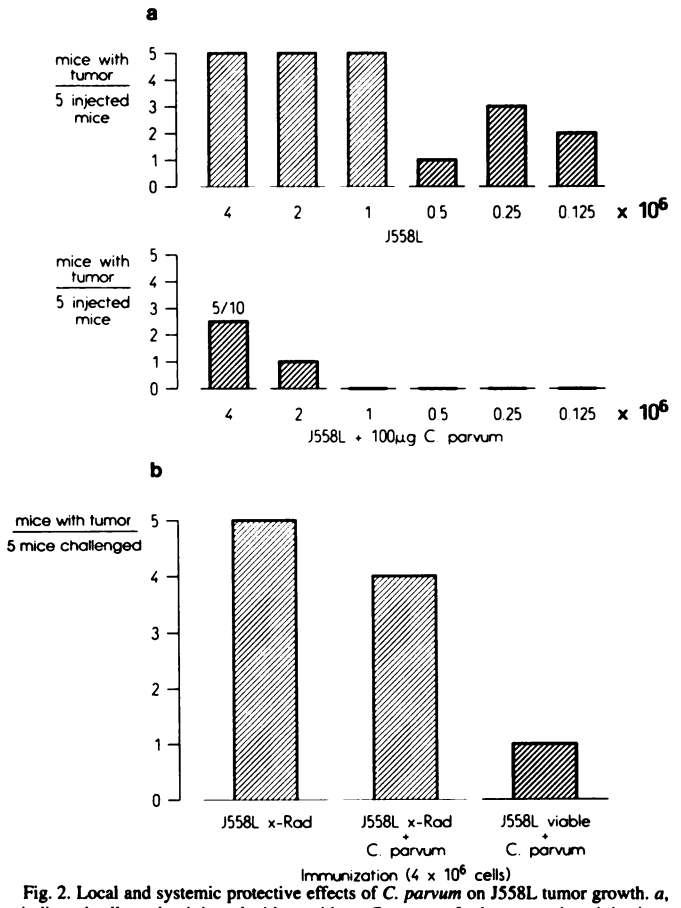

Fig. 2. Local and systemic protective effects of *C. parvum* on J558L tumor growth. *a*, an indicated cell number injected with or without *C. parvum*. In the group given injections of 4 × 10^6 cells plus *C. parvum* there were 10 mice. *b*, mice immunized by the indicated regimen contralaterally challenged with 4 × 10^6 J558L cells after 18 days.

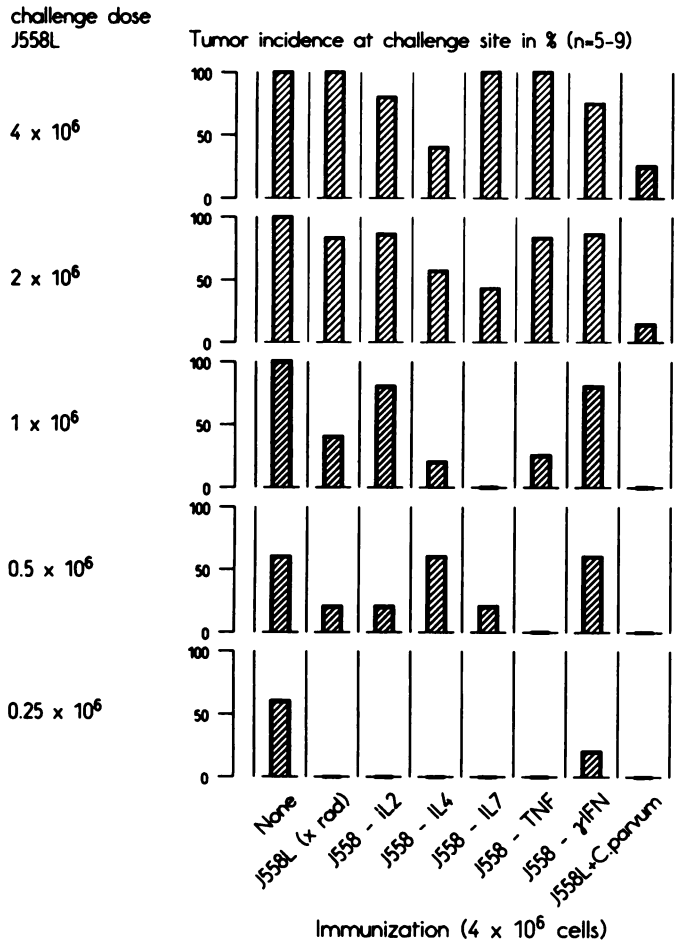

Fig. 3. Comparison of the immunogenic poteny of cytokine-producing J558L variants *versus* parental cells admixed with *C. parvum*. Mice were given s.c. injections of 4×10^6 of the indicated cells and contralaterally challenged after 3 weeks with the indicated J558L cell number.

Discussion

The potential to generate systemic immunity has been observed for tumor cells transfected with any of the 5 cytokines dealt with in this study (5–12). Therefore, we were surprised to find our cytokine-producing cell lines to be immunogens which were only slightly superior to irradiated cells not producing the cytokines. However, we do not think our data are necessarily in contrast to those of others with different tumor cell lines. The studies so far describing the generation of systemic immunity did not conclusively determine if this phenomenon was necessarily due to the secreted cytokines or might be explained solely by the exposure of viable tumor cells to the immune system during the primary rejection. Irradiated or mytomycin C-treated cells as controls were not included in those experiments (5–12). It should be noted, however, that in many cases well-characterized cell lines were used which are usually considered to be non-immunogenic, but even in this context the omission of irradiated cells as controls may be problematic because the immunogenicity of tumor cells may change with time (19). Moreover, dose escalations with challenges of tumor cells have not been performed in any of the reports describing the successful generation of systemic protective effects, allowing no assessment of the strength of the systemic response. The only reports which did use either mitomycin C-treated controls with IL-4-producing tumor cells (20) or included dose escalations with IFN-γ-producing tumor cells (21) are in agreement with our finding of a rather moderate systemic immune response. Of course, with other cytokines, cell lines, immunization regimens, or experimental systems designed to analyze the effect on small tumor burden or metastasis the results may be improved.

However, the observation that the tumor adjuvant *C. parvum* was even more effective in raising a protective response suggests that it may take further experimental studies to be able to judge whether and how this approach may best be used for the improvement of clinical vaccination trials.

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