

had a local recurrence, and all (47%) died from their tumour. The median progression interval was 24 months. After stratification by pathological grade, site, laterality, and number of nodes found at lymphadenectomy, there were no statistical differences in risk of disease progression or mortality between the two groups.

Transfer factor (TF)

At the end of this review, we have reserved a separate chapter to TF, since it has become matter of controversy, and yet it is still used with unequivocal success by several investigators, including ourselves.

TF has shown its clinical usefulness in the treatment of a variety of tumours, e.g. non-small-cell lung cancer (Kirsh et al., 1984; Fujisawa et al., 1983, 1984a, 1984b, 1996; Busutti et al., 1987; Whyte et al., 1992; Pilotti et al., 1996), Epstein-Barr virus-related tumours, i.e. nasopharyngeal carcinoma (Prasad et al., 1997) and Burkitt's lymphoma (Nkrumah et al., 1987; Neequaye et al., 1990), cervical cancer (Wagner et al., 1987) and hormone-unresponsive prostate cancer (Pizza et al., 1996).

In a prospective, not randomized trial Montie et al. (1977) used TF for the treatment of ten MRCC patients, and they reported temporary stabilization of metastatic disease. The same group of investigators treated 60 patients with MRCC in five different immunotherapy protocols consisting of a) TF, b) association of TF and BCG, c) association of TF, BCG, chloroethyl-cyclohexy-nitrosurea (CCNU) and megestrol acetate (Megase), d) association of BCG, CCNU, and Megase, and e) BCG alone (Montie et al., 1982). While this non-specific immunotherapy of renal adenocarcinoma has been associated with documented regression of metastases, response rates were similar to those obtained with hormonal therapy alone. Nonetheless, because of these results, further clinical studies were undertaken.

Thirty-seven MRCC patients, compared to 27 historical controls, were treated with combined immunotherapy including direct lymphatic injection of IL-2 and LAK cells, intramuscular injection of IFN- α -2a (10^6 units bi-weekly) and TF (bi-monthly injections of 4×10^8 mononuclear cell equivalent obtained from pooled buffy coats of healthy blood donors). This regimen produced CRs or PRs of metastases in 34% of the treated patients and stabilized progression of the disease in an additional 8%. The median survival was, respectively, 26 and 27 months for synchronous and metachronous metastatic treated patients, versus 8 and 14 months for the control group ($P < 0.001$) (Corrado et al., 1991). While no side effects were noticed in the treated patients, the observed results were comparable to those obtained by intravenous injections of large amounts of IL-2 and LAK cells, a protocol which nonetheless produced severe adverse side effects. Similar results have

been recently reported in 122 MRCC patients treated in a comparable way (Pizza et al., 2001) (Table 3).

However, despite over fifteen hundred reports proving activity *in vitro* and clinical usefulness in treating not only cancer, but also viral, fungal, and parasitic diseases, its total lack of toxicity, and the fact that no publication has ever challenged these claims (indeed, since animal models exist, it should be easy and inexpensive to document the failure to reproduce published evidence), TF has become controversial. The reason is simple: TF's characteristics (low molecular weight, undefined chemical structure, proteinaceous nature, but resistance to proteolytic enzymes and capability to survive oral administration), together with its unusual biological properties (non-species specificity, unconventional mode of action, e.g. transfer of antigen-specific information using infinitesimal amounts of active material) have generated fierce opposition. And the failure to unravel the molecular structure has led immunologists to doubt its very existence, following the precepts of today's consensual *good scientific behaviour practice* that one may sum up by: *discard facts rather than endanger the paradigm*, in other words deny anything missing a molecular explanation.

Thus, nearly fifty years after the first observations, TF remains an elusive and controversial entity, although biochemical studies have produced evidence that it is a small peptide, with possibly three ribonucleotides attached to it. However, its complete molecular identity is still elusive, and attempts to sequence the amino acids failed due to a blocked amino terminus. And yet, elementary logic tells us that ignorance of the chemical structure of a compound never curtailed its activity. For instance, when belladonna or aspirin were used as crude plant extracts, their properties and potency were the same as today when we know the precise structure of their active ingredients, i.e. scopolamine, atropine, I-hyosciamine or acetyl-salicylic acid.

But it seems that modern biomedical logic would rather that facts were dismissed and treatments inefficacious or toxic than incomprehensible. For it is more respectable to reject a fact than to be mixed up with a fluke. And yet, in the TF story, we are not dealing with a spooky phenomenon of the paranormal eliciting "*society's negative response, which leads individuals to suppress their experience for fear of rejection or ridicule*" (van Lommel et al., 2001), but with a clinically and experimentally well-documented reality.

Be that as it may, the clinical effects of TF deserve closer examination. For not only has this moiety the ability to instruct T lymphocytes to fight an intruder, but several publications suggest that it can also be used for prevention, i.e., as a vaccine soliciting the cell-mediated immunity that plays the most important role in combating neoplastic and viral diseases (Steele et al., 1980; Viza et al., 1986).

We are convinced that once its biochemical structure has been defined, the path to understanding its precise mode of action will be clear, consequently opening new avenues into the unknown in immunology and molecular biology. Recent work has already paved the way by partially unravelling the amino-acid sequence riddle, thus giving biochemical flesh to an elusive entity (Kirkpatrick, 2000).

Postface

Although several publications (Pizza et al., 1984, 1987, 1988; Lefesvre et al., 1987; Corrado et al., 1991) have shown since 1984 that it is possible to reduce the amount of the administered IL-2 without decreasing its efficacy, most clinicians clung to the high-dosage protocol, paying little attention to the alternative quasi-homeopathic amounts proposed by other investigators and, for that matter, to the patients' suffering. It seems that modern medical science has forgotten that catalytic processes are common in biochemistry, and that they require infinitesimal amounts of the catalyst to perform complex and sizeable reactions. Immunological interplay being part of life's chemistry, the range of IL-2 effective dose should have been fully investigated, particularly since it was published that 2 000–5 000 U of IL-2 trigger a remarkable cascade of immunological changes a few minutes after injection (Corrado et al., 1991). Indeed, the average standard treatment uses more than 500×10^6 U for a 3-month period vs. less than 10 000 U for the low-dose protocol, i.e. 50 000 times less!

In the past 15 years, we have treated 122 MRCC patients with intralymphatic injections of non-recombinant IL-2, LAK cells, IFN- α and TF. The results show a survival 3.5 times higher compared to that of historical controls. Furthermore, when the data were compared with those of similar studies, they showed an equivalent response rate (19.7%), but a better survival (45% at 3 years, 39% at 5 and 30% at 7), as well as a better median survival (28 months). Indeed, eleven years after the beginning of the treatment, the Kaplan-Meier curve shows a survival rate more than 25% (Pizza et al., 2001).

However, the most important advantage of this protocol is the fact that it is devoid of adverse side effects. In 1 692 intralymphatic administrations of 250–490 IL-2 units, in less than 2% of cases we observed chills, fever (less than 38°C), and/or hypotension. The 10 315 IL-2 inhalations caused dyspnoea, or hypertension (0.2%) in less than 2% of patients. Nonetheless, no medication was necessary, the side effects disappearing after discontinuing the IL-2 administration. Furthermore, the quality of life was not affected, and this is in agreement with the observations of Heinzer et al. (1999), who used inhaled IL-2.

In contrast, the IL-2 regimens currently employed in other centres induce severe adverse clinical side effects, as defined by the World Health Organization classification system (Miller et al., 1981). Thus, in "*recent studies, grade 3 and 4 toxicity has been observed in a substantial number of patients and 5.2–9.4% of patients died during the treatment periods, or within a month following the treatment, from causes unrelated to renal-cell carcinoma*" (Negrier et al., 1998).

Obviously, the scarcity of side effects in our patients is due to the low IL-2 dosage. In 14 years, we administered a total of 4 006 773 units of non-recombinant IL-2 to 122 MRCC patients. This amount is less than the amount of IL-2 that is usually administered in one day to one MRCC patient under the prevalent IL-2 protocols. A windfall but far from negligible result of our protocol is the substantial decrease of the cost of the treatment, i.e. from 7 000 € to 200 € for a 3-month period.

It is therefore obvious that long-term intra-lymphatic administration of IL-2 at a very low dose, together with LAK cells and TF, is not only safe, but it also produces significant clinical improvement and one of the best survival rates among those reported in the literature. Moreover, and most significantly, it offers a good quality of life, while it dramatically reduces the cost of treatment.

However, if today IL-2 appears to be the most effective form of immunotherapy for MRCC, further studies are warranted, since certain aspects of its mode of action are not totally understood. For instance, a faster progression of the disease is noticed when the treatment is interrupted.

A thorough evaluation requires a detailed study of the different immunotherapeutic agents, i.e. both natural and recombinant IL-2, administered with LAK intralymphatically together with or without inhaled and/or subcutaneous IL-2 at different doses. However, because of the relatively low incidence of this tumour and the need for investigation of several parameters, such study requiring the cooperation of several centres is complex and cumbersome. For example, although the cost of the LAK preparation is not high (less than 15 € per injection), it cannot be carried out on a routine basis, since it requires an important backup from a pathology laboratory. We would therefore like to suggest a simpler approach: evaluate the effect of intralymphatic injections of recombinant IL-2, together with subcutaneous and/or intranasal low-dose administration. This protocol should make the study easier, despite the fact that intralymphatic injection is not a routine practice, although better known today because of the use of lymphography.

It should be emphasized that the vaccination protocols, using both whole tumour cells *in vivo*, as well as dendritic cells expanded *in vitro* also hold great promise. In all probability, in the years to come they

will play an important role in future protocols of immunotherapy, most likely in association with existing and new immunostimulating agents. The fight against cancer is far from being over, and the research for the development of new tools is of the essence.

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