Biological Response Modifiers

43

INTERFERON ALPHA ENHANCES THE CYTOTOXICITY OF CYTOSINE ARABINOSIDE IN HL60 CELLS BY INCREASING APOPTOSIS AND IS SYNERGISTIC WITH CYTOSINE ARABINOSIDE IN CLINICAL COMBINATION TREATMENT REGIMENS. Bezwoda WR, Seymour L, Mansoor N. Department of Medicine, University of the Witwatersrand Medical School, 7 York Road, Parktown 2193, Johannesburg, South Africa.

In-vitro synergy between IFNα and Ara-C was demonstrated in HL60 cells. Amounts of IFNα as low as 10 μ/ml potentiated the cytotoxic effects of Ara-C and moreover prevented recovery from sublethal toxicity following short duration exposure to Ara-C. The cytotoxic effect was shown to be due to an increase in apoptotic cell death.

Following on the in-vitro studies a clinical study of this combination was undertaken. Twenty four patients with relapsed refractory hematologic malignancy were treated with a combination of cytosine arabinoside (100 mg/m^2/day x 10 days) and IFNα (10 million U/m^2/day x 10 days) given by continuous i.v. infusion. Eleven patients achieved CR including 2/6 with relapsed refractory AML, 7/9 with blastic CML, 1/3 high grade NHL and 3/4 with follicular lymphomas. There were also 2 patients with RAEB who achieved a complete hematologic response. In-vitro studies utilising leukaemic blast cells from patients with AML suggest that the mechanism of effect is similar to that observed in HL60 cells. This regimen appears to be a promising approach for patients with hematologic malignancies.

44

INTRAPLEURALrIL-2 IN PLEURAL EFFUSIONS DUE TO MALIGNANT MESOTHELIOMA.

Castagneto B., Botta M., Degiovanni D., Bretti S., Mutti L.

Specific object of this study was to evaluate the toxicity and the efficacy of intrapleural r IL-2 in the treatment of malignat pleural effusions due to pleural mesotelioma.
The schedule of administration was: rIL-2 at the dose of 9x10^6 IU on day 1 and 2 every 7 days. The administration was repeted for 4 weeks. The results were evaluated according to the criteria of Paladine et Al. Eleven patients entered the study. An objective response was seen in 10/11 (90.9%). The toxicity observed was moderate and included fever and flu-like sindrome in 9/11 (81.8%). All other side effects were mild.
This study shows that intracavitary administration of IL-2 is an effective palliative treatment of the pleural effusions due to malignant mesothelioma. The treatment is also well tolerated.

Castagneto Bruno. Department of Oncology. S.Spirito Hospital. Viale Giolitti n.2, Casale Monferrato (AL) 15033 Italy
Telef.: 0142 / 434255 FAX:0142 / 434390.

45

S. Duggan, H.P. Redmond, J McCarthy, R.W.G.Watson D. Bouchier-Hayes. Dept. of Surgery, Beaumont Hospital, Dublin

RESIDENT MACROPHAGES DO NOT REQUIRE DUAL SIGNAL STIMULATION FOR TUMOUR CELL LYSIS Inflammatory macrophages (IMØ) require a dual signal stimulus to induce tumour cell killing. However, observations in this laboratory suggested that a single signal would suffice for activation of resident MØs.(RMØ) The aim of this study was to assess the requirement of single vs dual signal stimulation for the induction of murine RMØ and IMØ cytotoxicity. RMØs were harvested from control CD-1 mice and IMØs were harvested 72hrs post injection of proteose peptone.MØs were stimulated in-vitro for 24 hours with LPS (1ug/ml) ± IFN-g(10^{3}U/ml). Cytotoxicity, TNF, O2-(superoxide anion) and cytosolic arginase activity were assessed as markers of cytotoxic activity. Maturation/activation was assessed by CD11b cell surface expression.

	Cytotoxicity %		O2- nmols/μg BSA		TNF pg/μgBSA		Arginase μmols/μg BSA/hr	
	RMØ	IMØ	RMØ	IMØ	RMØ	IMØ	RMØ	IMØ
Control	27±1	33±1	.019±0	.021±0	4±1	7±2	30±9	27±8
IFN-g	85±10*	41±2	.034±0*	.022±0	29±4*	10±2	83±6*	43±7
LPS	61±9*	37±3	.031±0*	.021±0	23±3*	8±3	61±11*	33±6
LPS+IFN-g	71±5*	74±4*	.028±0*	.033±0*	23±5*	30±5*	73±6*	81±3*

Data=Mean+SD Significance set at *P<0.05. RMØs stimulated with either IFN-g or LPS as single signals had enhanced cytotoxicity compared with same group controls and IMØs. These results correlated closely with TNF, O2- and arginase values obtained.CD11b expression MCF was 276±6=RMØ vs 65±5=IMØ. These results indicate that a single stimulatory signal is sufficient to enhance cytotoxicity by RMØ, whereas a dual signal is a prerequisite for upregulation of tumouricidal activity by IMØ.

46

S. Duggan, H.P. Redmond, J. Mc Carthy, D. Bouchier-Hayes.Dept. of Surgery, Beaumont Hospital, Dublin. THE CYTOTOXIC POTENTIAL OF TIMS IS MEDIATED THROUGH ENHANCED ARGINASE PRODUCTION.

Activated macrophages (MØs) produce superoxide anion (O2-)and tumour necrosis factor (TNF), known tumouricidal mediators. Paradoxically, tumour infiltrating MØs (TIMs) have been reported to demonstrate impaired production of these mediators, thus casting doubt on an anti-tumour role for TIMs. Solid tumours are known to contain high levels of the enzyme arginase, however, the source is unclear. We postulated that an important source of arginase is in fact TIMs and enhanced production of the cytotoxic enzyme may contribute to TIM tumouricidal activity. Human monocytes (Mø) were isolated from blood pre-operatively obtained from patients (n=5) undergoing surgery for colorectal cancer. Colorectal tumour specimens 5-10gm were freshly procured and digested with trypsin (10x), DNAase (20μg/gm) and collagenase (20μg/gm). The percentage of TIMS was then assessed by cytocentrifugation, and both Møs and TIMS were seeded at a concentration of 2 x 10 5 /well. Cytotoxicity was assessed using a standard Cr 51 release assay against the Wehi 164cell line,TNF, O2- generation and cytosolic arginase activity were also measured.

	Cytotoxicity %	Superoxide nmols/mg BSA	TNF pg/mg BSA	Arginase mmols/mg BSA/hr
B	76.20±9.36	0.020±.003	15.80±2.02	91.20±17.04
TIM	93.60±2.61*	0.016±.003*	12.34±1.53*	154.0±17.28*

B=peripheral blood Møs; Data = Mean±SD, Stats.= Students t-test,*p<.05, PWe have confirmed that TIMs have indeed impaired production of TNF and O2-, however, their cytotoxicity is enhanced and is associated with an increase in arginase activity. TIM derived arginase contributes to enhanced cytotoxicity therefore the arginase-dependent polyamine-biosynthetic pathway merits further evaluation.