Anti-Cancer Drugs 1995, **6**, pp. 250–258

Colorectal cell line suppression of lymphokine activated killer cell generation is reversed by suramin

Paul D Allen,[1] David H Johnston,[2] Marion G Macey,[1] Norman S Williams[2] and Adrian C Newland[1]

Departments of [1]Haematology and [2]Surgery, The London Hospital Medical College, Turner Street, Whitechapel, London E1 2AD, UK. Tel: (+44) 71 377 7000 extn 3056; Fax: (+44) 71 377 7677.

Progressive tumor growth is associated with a state of immunosuppression. One mode of immunosuppression is thought to be mediated by immunosuppressive factors of tumour origin. We have investigated *in vitro* the possibility that suramin could be used to blockade tumor derived suppressor factors and enhance the effectiveness of rhIL-2 therapy. These data show that factors derived from cultures of the colorectal carcinoma cell line LoVo suppressed the percentage of cells expressing the natural killer cell antigen CD56 in 92% of individuals and the cytotoxic T cell antigen CD8 in 77% of individuals tested from a panel of 13 normal healthy volunteers. Suramin at 200 μg/ml restored the percentage of cells expressing CD56 to levels higher than the control cultures and reduced the suppression in those expressing CD8 to non-significant levels. LoVo produced factors also suppressed the expression of the activation associated antigens CD25, CD71 and HLA-Dr with suramin restoring CD25 expression but not CD71 or HLA-Dr. Functional studies using ^{51}Cr-release assays showed that LoVo produced factors could suppress cytotoxicity in 46% of individuals tested, and of these a reduction in suppression by suramin was demonstrated in 50% of individuals against Daudi target cells and 33% against K562 target cells.

Key words: IL-2, reversal, suppressor factors, suramin

Introduction

Treatment of peripheral blood lymphocyte populations with recombinant human (rh) IL-2 *in vitro* results in proliferation of natural killer (NK) cells and T lymphocytes. There is also a concomitant generation of lymphokine activated killer (LAK) cell activity manifest as an increased cytotoxic activity of the lymphocyte effector population towards standard target cell lines.[1] Intravenous administration of rhIL-2 also induces a LAK response *in vivo*. A variety of experimental treatment regimens which achieve induction of this response either *in vivo* or by a combination of *in vivo* and *in vitro* techniques have resulted in clinical responses and complete remissions in some patients with certain types of otherwise untreatable advanced solid malignancies.[2,3] Although patients suffering from metastatic renal carcinoma and disseminated melanoma have shown an encouraging response rate to a range of rhIL-2-based treatment protocols, those with adenocarcinomas arising from mucin secreting glandular epithelial organs, such as breast, colon and pancreas, have proven to be less amenable to rhIL-2 based therapies.[4] It has been previously suggested that some tumors may elaborate substances which inhibit the functional activity of LAK cells as measured by their cytotoxicity towards standard tumor target cells.[5] A broad spectrum of substances including cytokines, growth factors[6] and other partially characterized substances have been implicated in LAK cell suppression by *in vitro* experimentation.[7,8] We have also shown that products of the colorectal cell line LoVo are antiproliferative towards precursors of LAK cell generation, and suppress both activation associated antigen expression and *in vitro* LAK cell function.[9] Therefore, blockade of these deleterious substances may enhance the effectiveness of rhIL-2-based therapies.

Suramin is a polysulfonated aromatic compound with a net anionic charge originally introduced for the treatment of trypanosomiasis and onchocerciasis.[10] There has been renewed interest in this drug after it was shown to be a potent inhibitor of retroviral reverse transcriptase[11] and to protect $CD4^+$ cells from infection and the cytopathic effects of the human immunodeficiency virus (HIV).[12] The physicochemical properties of the suramin molecule enable it to bind to and block the activity of a wide range of enzymes, including those of the complement and coagulation systems.[13] More recently, suramin has been shown to bind to or displace certain growth factors from their respective receptors. These include platelet derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor (TGF)-β, members of the fibroblastic growth factor (FGF) family, heparin binding growth factor type 2 and insulin growth

Correspondence to PD Allen

factor 1.[14] It causes growth inhibition of a variety of solid tumor cell lines[15,16] and some but not all lymphoid cell lines.[17] Although toxicity appears to reduce its usefulness as an agent against HIV[18] it is currently being used in clinical trials against malignant diseases.[19]

These combined results prompted us to investigate the possibility that suramin could act as an adjuvant to rhIL-2-based immunotherapy by blockading suppressor factors released by the colorectal cell line, LoVo. We report the effects of suramin on LoVo induced suppression of LAK effector cell populations, activation associated antigen expression and LAK cell function.

Materials and methods

Culture conditions

Peripheral blood samples were obtained from 13 normal healthy volunteers using a 1 in 10 dilution of 3.13% sodium citrate as anticoagulant. Peripheral blood mononuclear cells (PBMCs) were collected after Ficoll-Hypaque density gradient centrifugation (Lymphoprep; Nycomed, Norway). These cells were seeded at a density of 10^6/ml in 5 ml volumes into a 50 ml plastic flask (Nunc). Four cultures were initiated from each sample: (i) control cultures containing RPMI (Gibco) with 10% heat inactivated fetal calf serum (Gibco), L-glutamine (2 mM) (Sigma), penicillin (100 U/ml) and streptomycin (100 μg/ml), (ii) as above but with the addition of rhIL-2 (Proleukin; Eurocetus, The Netherlands) at a final concentration of 1000 U/ml, (iii) as in (ii) with 20% of the medium by volume containing medium conditioned by exposure to the colorectal cell line LoVo as described below (rhIL-2 was added at a final concentration of 1000 U/ml) and (iv) as in (iii) but with the addition of 200 μg/ml suramin (Germanin; Bayer, Germany), rhIL-2 was again present at 1000 U/ml. All flasks were incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 96 h prior to phenotypic and functional analysis.

The colonic adenocarcinoma cell line LoVo was obtained from the European Collection of Animals Cell Cultures (ECACC, Porton Down, UK) and maintained mycoplasma free (screened by Hoechst stain) in RPMI as described in (i). Conditioned medium from the LoVo cell line was obtained by washing a confluent monolayer of LoVo cells, cultured in a 50 ml flask, three times in fresh culture medium. Supplemented RPMI medium minus fetal calf serum (20 ml) was incubated with the monolayer for 24 h. This medium was then harvested and used in the rhIL-2 activation cultures, as described above, at a concentration of 20% by volume.

Phenotypic and functional analysis

In this study CD3 was used to identify mature T cells, and CD4 and CD8 to identify T helper and T suppressor/cytotoxic subsets, respectively. CD56 was used to assess NK cell numbers. Lymphocyte activation was assessed by measuring changes in the expression of the IL-2 receptor α chain (CD25), the transferrin receptor (CD71) and HLA-Dr. All antibodies were FITC-conjugated (Becton Dickinson), except for the anti HLA-Dr antibody which was phycoerythrin-conjugated (Coulter), and all were used at 10 μl/10^6 cells. Cells were incubated with antibody for 30 min at 4°C, washed twice and analyzed on an Epics C flow cytometer (Coulter Electronics). Percentage positive cells were assessed by the increase in fluorescence over and above the background of isotype controls.

Cytotoxicity assays were performed as previously described.[20] Effector cells were taken from the cultures described above, washed twice and incubated with ^{51}Cr-labeled target cells (K562 and Daudi) at an effector target ratio of 12:1 for 4 h in RPMI plus 10% fetal calf serum, L-glutamine and antibiotics. Each assay was carried out in triplicate. The percentage lysis in each assay was determined using the formula: % cytotoxicity = (test c.p.m. − spontaneous c.p.m.)/(total c.p.m. − spontaneous c.p.m.) where test c.p.m. is the mean c.p.m. released from target cells in the presence of effector cells, spontaneous c.p.m. is the mean c.p.m. released by target cells alone and total c.p.m. is the mean c.p.m. released by targets lysed with detergent.

Statistics

Data has been analyzed using the Student's paired *t*-test, where $p < 0.05$ was considered significant.

Results

Effects of suramin on LoVo modulation of rhIL-2 induced changes in lymphoid subset antigen expression of PBMCs

Across the panel of individuals, the mean percentage antigen expression showed that the addition of

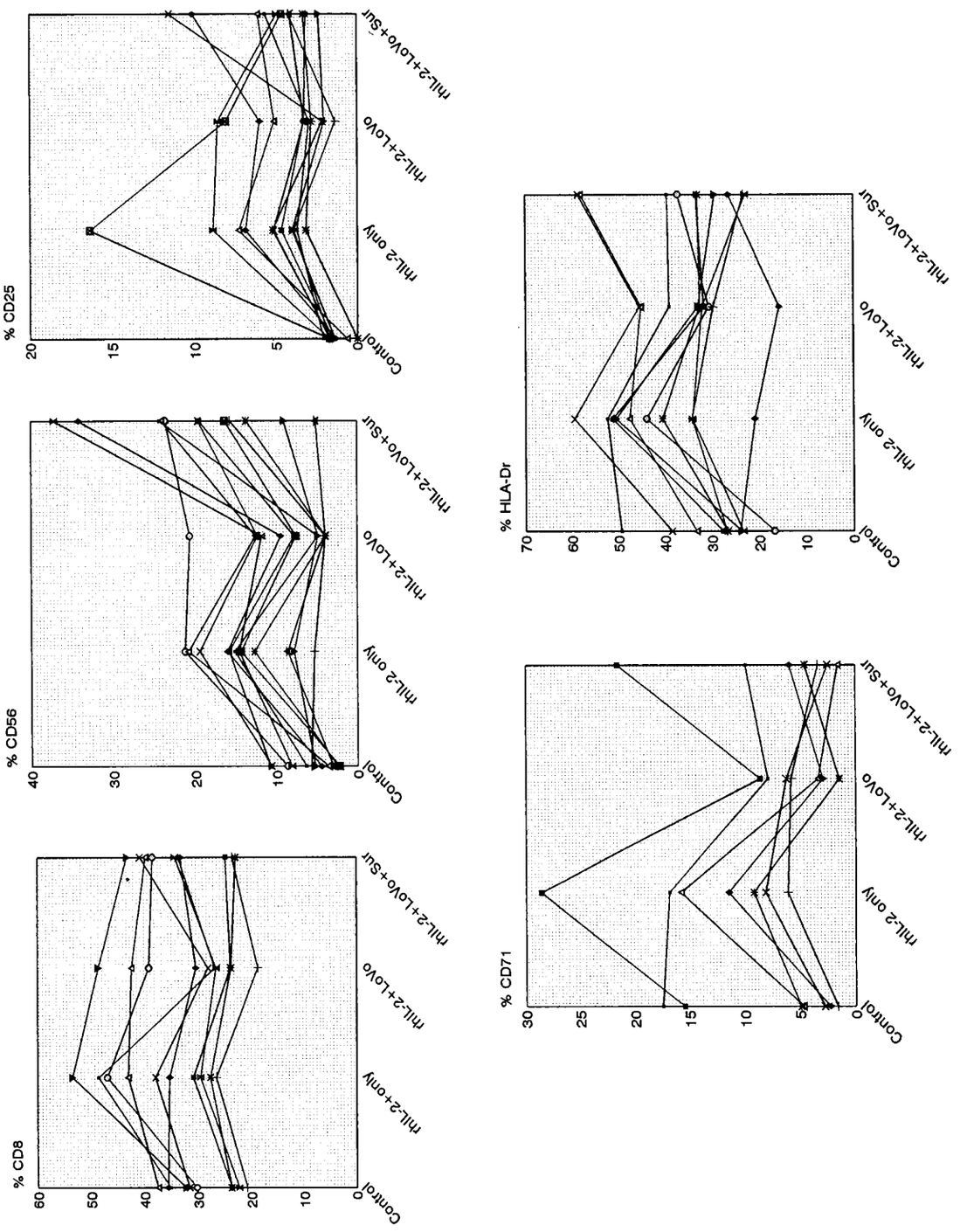

Figure 1. The effect of 200 μg/ml suramin on the percentage expression of CD8, CD56, CD25, CD71 and HLA-Dr in PBMCs from individuals who responded to the suppressive nature of LoVo cell line conditioned medium. The augmentation of these parameters due to rhIL-2 activation is also shown.

rhIL-2 induced a 2-fold increase in $CD56^+$ cells ($p < 0.001$, $n = 13$) and an augmentation of cells expressing CD3 ($p = 0.004$, $n = 9$). This latter observation was brought about by an increase in those expressing CD8 ($p = 0.014$, $n = 13$) and not those expressing CD4. Addition of 20% by volume of LoVo conditioned medium resulted in a suppression of the rhIL-2 induced augmentation of $CD8^+$ lymphocytes ($p = 0.025$, $n = 13$) and the rhIL-2 induced increase in cells expressing CD56, which approached significance ($p = 0.088$, $n = 13$). There were no changes due to LoVo conditioned medium in cells expressing either CD3 or CD4 in this panel of individuals. A closer look at the data reveals a difference in response to stimuli by different individuals. The increase in cells expressing CD8 due to rhIL-2 was seen in nine of the 13 individuals screened ($p = 0.001$, $n = 9$). There was no response to rhIL-2 by one individual and a small fall in $CD8^+$ cells was seen in three individuals. The proportion of cells expressing CD56 was augmented by rhIL-2 in 12 of the 13 individuals ($p < 0.001$, $n = 12$). Addition of 20% LoVo supernatant to rhIL-2 containing cultures caused suppression of $CD8^+$ cells in 10 of the 13 individuals ($p = 0.008$, $n = 10$). There were no significant changes in the other three individuals. However, the presence of suramin in parallel cultures of the same 10 individuals reduced the suppression of $CD8^+$ cells by LoVo supernatants to non-significant levels when compared with cultures containing rhIL-2 only. Similarly, addition of LoVo supernatants caused a suppression of rhIL-2 enhancement of $CD56^+$ cells in 12 of the 13 individuals ($p < 0.001$, $n = 12$). Addition of suramin to parallel cultures of these 12 individuals resulted in reversal of the LoVo induced suppression and the subsequent enhancement in mean percentage of $CD56^+$ cells to levels above that of cultures containing only rhIL-2 ($p = 0.024$, $n = 12$) (Figure 1).

Suramin also induced a 50% fall in the proportion of cells expressing CD4 ($p < 0.001$), which we have reported previously (data not shown).[21]

Effects of suramin on LoVo modulation of rhIL-2 induced changes in activation associated antigen expression on PBMCs

Twelve donors from the panel were screened for changes in activation associated antigen expression. Overall, augmentation of the mean percentage of cells expressing CD25 ($p = 0.001$), CD71 ($p = 0.001$) and HLA-Dr ($p < 0.001$) was seen in cells cultured in the presence of rhIL-2 when compared with control cultures. rhIL-2 induced rises in activation associated antigen expression were suppressed to a significant degree with respect to CD25 ($p = 0.014$) and HLA-Dr (p=0.006), and approached significance for CD71 ($p = 0.059$) by the presence of 20% by volume of LoVo supernatant.

Eleven of the 12 individuals responded to the suppressive effects of LoVo supernatants with respect to CD25 expression ($p = 0.009$, $n = 11$). The addition of suramin to the same 11 cultures reduced the LoVo induced suppression to non-significant levels when compared with cultures containing rhIL-2 only. LoVo supernatants caused a drop in percentage of cells expressing CD71 in seven of the 12 individuals tested ($p = 0.014$, $n = 7$), but suramin had no significant effect on this suppression, even though some individuals did respond. Similarly, the rhIL-2 induced augmentation of HLA-Dr was suppressed by LoVo products in 10 of the 12 volunteers studied ($p = < 0.001$, $n = 10$) but suramin again had no statistically significant effect on this suppression (Figure 1).

Effects of LoVo supernatants on rhIL-2 induced changes in cytotoxicity and its modulation by suramin

The mean percentage cytotoxicity of all 13 individuals tested was increased by rhIL-2 against both Daudi and K562 target cells. Of these 13, six showed a suppression of cytotoxicity due to LoVo produced factors against Daudi ($p = 0.001$, $n = 6$) and K562 ($p = 0.009$, $n = 6$) target cells. Seven of the 13 gave a small additional increase in cytotoxicity, above that induced by rhIL-2 alone, when LoVo supernatant was added (Figure 2).

Of the six out of 13 individuals where LoVo produced factors caused a suppression of rhIL-2 action, the addition of suramin reversed the LoVo induced suppression in three of the six using Daudi target cells and in two of the six using K562 as target cells. An additional decrease in cytotoxicity observed due to the presence of suramin in cultures containing rhIL-2 and LoVo products seen in three of the six individuals when using Daudi cells as targets and in four of the six when using K562 as target cells was not significant in either case (Figure 2). To show that the functional variation in response to LoVo supernatant and suramin is due to differences in response by different individuals and not assay variability, two donors from the panel were selected for further investigation. LoVo supernatant suppressed rhIL-2 enhanced cytotoxicity in five of five assays against

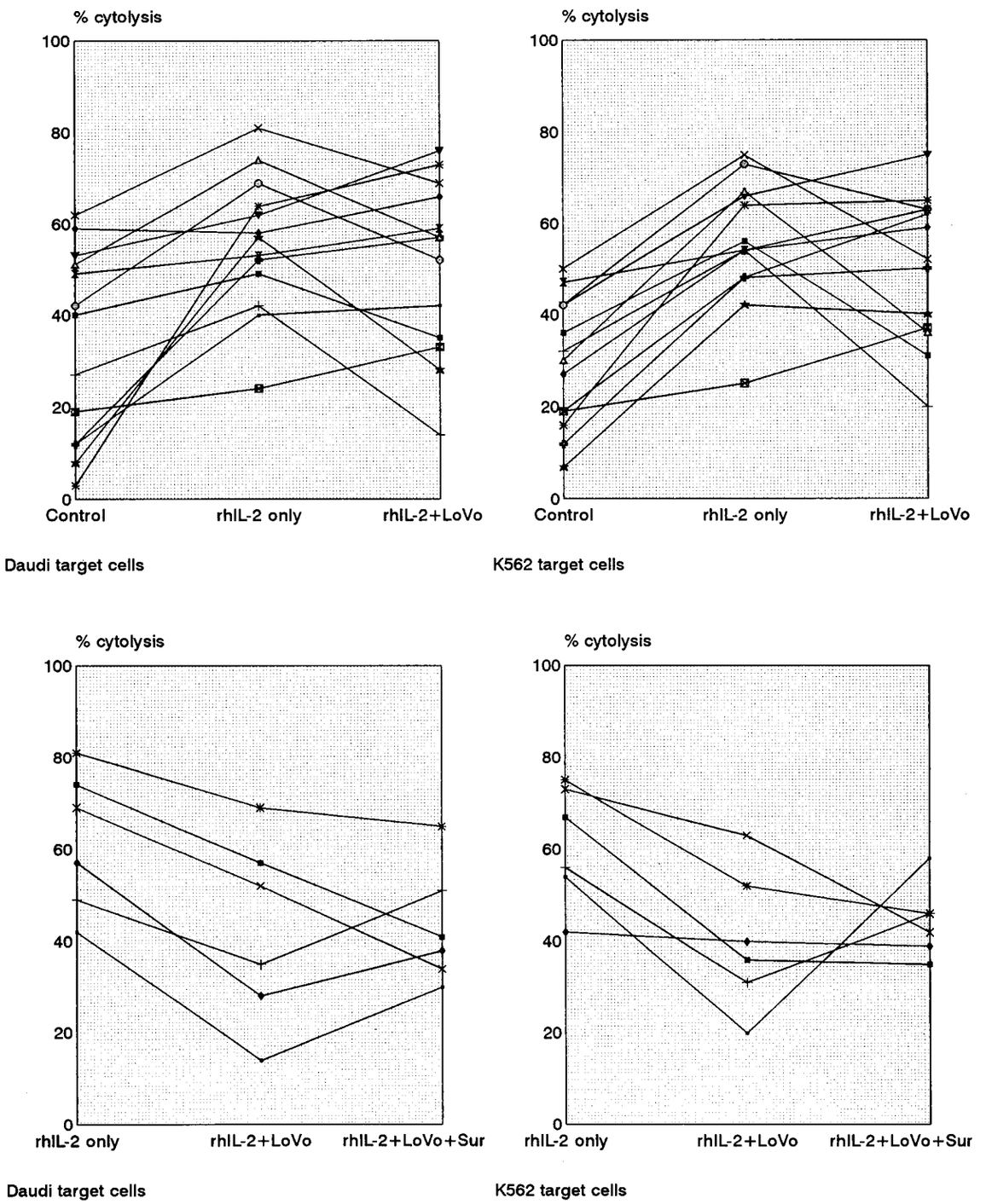

Figure 2. Top panel: the effect of LoVo cell line conditioned medium on the cytolytic ability of PBMCs isolated from the panel of 13 normal individuals, against Daudi and K562 target cells using a ^{51}Cr-release assay. Bottom panel: the modulatory effect of 200 μg/ml suramin on the cytolytic ability of PBMCs isolated from normal individuals who responded to the suppressive nature of LoVo cell line conditioned medium.

Daudi ($p = 0.007$, $n = 5$) and K562 ($p = 0.017$, $n = 5$) target cells and 200 μg/ml suramin reversed the suppression to non-significant levels in all experiments (Figure 3). In a second individual, LoVo supernatants suppressed rhIL-2 enhanced cytotoxicity in three of three assays but suramin failed to reverse this suppression in all three experiments (data not shown).

In control cultures containing rhIL-2 and suramin only, suramin was shown to have no effect on rhIL-2 augmentation of cytotoxicity against either Daudi or K562 target cell lines.

Control cultures

Throughout the experiments described above, suramin had no significant effect on expression of any of the antigens discussed hitherto or on cytotoxicity, either in the presence or absence of rhIL-2 except for the expression of CD4, which was always significantly lower after 96 h incubation with suramin.[21]

Discussion

Despite evidence of rhIL-2 activation of NK cells, cytotoxic T lymphocytes (CTLs) and macrophages *in vitro*, and the eradication of experimentally induced tumors in rodent models, the efficacy of immunotherapy has remained poor. There is evidence to show that progressive tumor growth is accompanied by a concomitant immunosuppression irrespective of tumor location or etiology. It is also clear that the suppression is directly or indirectly dependant on the presence of tumor.[22] Immunosuppression by tumor is particularly evident with large tumors, even when ideal conditions of immunogenic tumor antigens and susceptibility to effector cell lysis prevail.[23] One mode of immunosuppression is thought to be mediated by a range of tumor produced substances, including growth factors and cytokines. These include TGF-β[6], IL-4,[24] prostaglandins (25) and certain factors characterized by molecular weight only.[7,8] Therefore a blockade of these factors should enhance the effectiveness of rhIL-2 therapy. We investigated the possibility that suramin could act in this capacity.

The complexity of action of suramin and its multiplicity of target molecules renders its precise mechanism of inducing antiproliferative effects difficult to determine. However, a consensus of opinion suggests that its primary function is to block growth factors essential for tumor survival and their respective receptors. Its reported antitumor effects could well be mediated by disruption of autocrine growth factor loops which some tumors are thought to use to promote their own growth.

In clinical trials on patients with adrenocortical carcinomas, renal carcimomas, prostatic cancer and acquired immunodifficiency syndrome (AIDS),[18] it

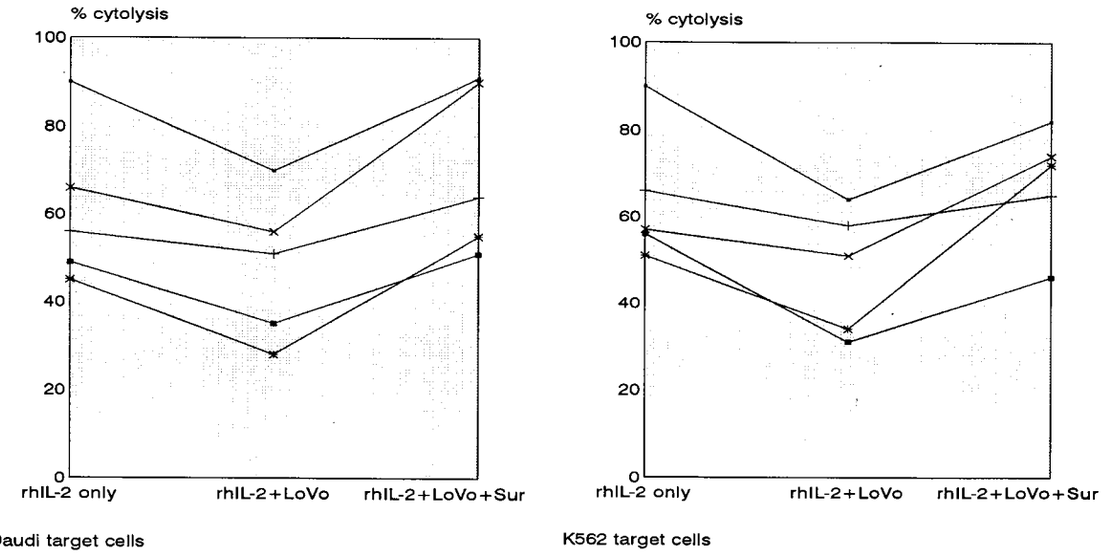

Figure 3. LoVo suppressed cytotoxicity against Daudi (61.20 ± 17.96–48.00 ± 16.78%, $n = 5$, $p = 0.007$) and K562 (64.00 ± 15.51–47.60 ± 15.51%, $n = 5$, $p = 0.017$) target cell lines in five out of five experiments conducted on PBMCs isolated from a single individual. Suramin reversed this suppression in all five experiments to non-significant levels (Daudi: 61.20 ± 17.96 versus 70.20 ± 19.12, $n = 5$, $p = 0.094$; K562: 64.00 ± 15.51 versus 67.80 ± 13.61, $n = 5$, $p = 0.585$).

has become clear that a major obstacle to suramin therapies is its toxic effects when serum levels exceed 300 μg/ml. This makes the maintenance of a serum suramin therapeutic window of 150–300 μg/ml essential. It is for this reason that we used suramin at a concentration of 200 μg/ml throughout our experiments.

We have previously reported the suppressive nature of LoVo conditioned medium.[9] This study provides evidence that suramin will reverse the suppression of rhIL-2 activation of PBMCs by LoVo conditioned medium.

In our panel of PBMC cultures from 13 individuals, rhIL-2 imparted a proliferative advantage for cells expressing the NK cell antigen CD56 and the CTL antigen CD8. There was a suppressive response to LoVo produced factors in 92% of cultures with respect to CD56 expression and in 77% of cultures with respect to CD8. Where LAK effector cell phenotypes were suppressed, the additional presence of suramin caused a reversal of these effects in 100% of cases. Furthermore, the presence of suramin in cultures containing LoVo conditioned medium enhanced the population of cells expressing CD56 to levels in excess of the control cultures containing rhIL-2 only. Suramin did not have a similar enhancing effect on CD56 percentage expression in control cultures of PBMCs containing only rhIL-2 and suramin, implying a factor present in LoVo supernatants which enhances CD56 proportions if either itself or another factor is removed by suramin.

Activation of both NK cells and T lymphocytes by rhIL-2 is associated with increased expression of the rhIL-2 receptor α chain CD25,[26] CD71 (transferrin receptor)[27] and the major histocompatibility class II antigens, HLA-Dr.[28] These data confirm that rhIL-2 enhances the expression of each of these activation associated antigens. The presence of LoVo products reduced the enhancement in expression of CD25, CD71 and HLA-Dr in 92, 67 and 92% of the cultures, respectively. Suramin restored CD25 expression in all cultures in which it had been suppressed, but it had no overall effect on the proportion of cells expressing CD71 or HLA-Dr. Dividing cells require iron which is delivered by the CD71. However, cells expressing CD71 are not neccessarily in a proliferative state, since some cells express CD71 which cannot divide.[29] Therefore CD71 is probably more closely related to intracellular Fe^{2+} requirements rather than proliferation or activation induced by rhIL-2.

There was an augmentation of cytolysis in 100% of the cultures incubated with rhIL-2 against both Daudi and K562 target cells. Only 46% of the cultures responded to LoVo produced products and, of these, suramin reversed the suppression to non-significant levels in 50% of the suppressed cultures against Daudi cell targets and 34% against K562. However, in a single individual shown to be susceptible to LoVo supernatant suppression, suramin reversed the suppression in five successive assays. Similarly, failure to restore function in a second individual was also found to be a repeatable observation.

The variablity seen in individuals towards suppression of cytotoxicity by LoVo produced factors and the subsequent reversal or non-reversal by suramin probably reflects the complex network of cytokines in the effector/target cell environment and during LAK cell generation.

For example, TGF-β is produced by a range of tumor cell types[30] as well as rhIL-2 generated LAK cells[31] and is readily bound by suramin.[14] It has been shown to inhibit LAK cell generation *in vitro*, resulting in a reduced cytolytic capability and a concomitant reduction in CD25 expression. Secondary cytokine production of tumor necrosis factor (TNF)-α and IFN-γ was also impaired.[22] TGF-α induced suppression of cytotoxicity was reversible by exogenous addition of TNF-α and IFN-γ.[33] Moreover, TGF-β suppression of CTL development in mixed lymphocyte cultures was also reversed by addition of TNF-α,[34] suggesting that a balance between TNF-α and IFN-γ, on one side, and TGF-β, on the other, could determine whether cytotoxicity or its inhibition prevails. IL-4 also suppresses IL-2 induced cytotoxicity with an associated suppression of CD25 expression. Again these effects proved reversible by addition of exogenous TNF-α and IFN-γ.[33]

Therefore, whether cytolysis of target cells occurs or not will depend upon the balance of cytokines mentioned above and other factors, e.g. prostaglandins. Moreover, the proportions of these factors will influence the effect of suramin on whether cytotoxic conditions can be maintained.

It has been shown that suramin can bind rhIL-2, at concentrations considerably higher than those used here and thus block rhIL-2 activity.[35] However, our control cultures show suramin had no deleterious effects on rhIL-2 activity at 200 μg/ml; a serum concentration with no toxic effects.

Conclusion

These results show that suramin maintains or enhances lymphoid cell populations which constitute

the effector cell populations of the rhIL-2 induced LAK phenomenon in the presence of factors produced by a colorectal adenocarcinoma cell line known to be antiproliferative towards these cell populations. Also, it can maintain CD25 levels in the presence of these factors and will reverse the suppressive nature of these substances in functional studies on PBMCs isolated from responsive individuals. We suggest that suramin has the potential to block immunosuppressive factors released by tumors. Using suramin in combination with rhIL-2 based immunotherapy regimens would represent a novel use for this anticancer agent.

References

1. Grimm EA, Mazumber A, Zhang HZ, *et al.* Lymphokine activated killer cell phenomenon: lysis of natural killer cell resistant fresh solid tumour cells by interleukin-2 activated autologous human peripheral blood lymphocytes. *J Exp Med* 1982; **155**: 1823–41.
2. Rosenberg SA, Lotze MT, Muul LM, *et al.* Observations on the systemic administration of autologous lymphokine activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *New Engl J Med* 1985; **313**: 1485–92.
3. Topalian SL, Soloman D, Avis FP, *et al.* Immunotherapy of patients with advanced cancer using tumour infiltrating lymphocytes and recombinant human interleukin 2: a pilot study. *J Clin Oncol* 1988; **6**: 839–53.
4. Rosenberg SA, Lotze MT, Yang JC, *et al.* Experience with the use of high dose interleukin 2 in the treatment of 652 cancer patients. *Ann Surg* 1989; **210**: 474–85.
5. Nelson M, Nelson DS. Inhibition of cell mediated immunity by tumour cell products: depression of interleukin-2 production and responses to interleukin 2 by mouse spleen cells. *Immunol Cell Biol* 1988; **66**: 97–104.
6. Grimm EA, Crump WL, Durett A, *et al.* TGF-β inhibits the *in vitro* induction of lymphokine activated killing activity. *Cancer Immunol Immunother* 1988; **27**: 53–8.
7. Nelson M, Bremner JAG, Nelson DS. Tumour cell products inhibit both functional immunoreactive interleukin 2 production by human blood monocytes. *Br J Cancer* 1989; **60**: 161–3.
8. Ebert EC, Roberts AI, O'Connell SM, *et al.* Characterisation of an immunosuppressive factor derived from human colon cancer cells. *J Immunol* 1987; **138**: 2161–8.
9. Johnston DH, Allen PD, Newland AC, *et al.* Inhibition of proliferation, activation and function of human natural killer cells *in vitro* by secreted products of LoVo, a colorectal cancer cell line. *Int J Oncol* 1993; **3**: 763–8.
10. Hawkin F. Suramin, with special reference to onchocerciasis. *Adv Pharmacol Chemother* 1978; **15**: 289–322.
11. De Clerq E. Suramin—a potential inhibitor of reverse transciptase of RNA tumour viruses. *Cancer Lett* 1979; **8**: 9–12.
12. Mitsuya M, Popovic M, Yarchoan R, *et al.* Suramin protection of T-cells *in vitro* against infectivity and cytopathic effect of HTLVIII. *Science* 1984; **226**: 172–4.
13. Eisen V, Loveday C. Effects of suramin on complement, blood clotting, fibrolysis and kinin formation. *Br J Pharmacol* 1973; **49**: 678–87.
14. Voodg TE, Vansterkenburg ELM, Wilting J, *et al.* Recent research on the biological activity of suramin. *Pharmacol Rev* 1993; **45**: 177–203.
15. Fantini J, Rognoni JB, Roccabianca M, *et al.* Suramin inhibits cell growth and glycoloytic activity and triggers differentiation of human colic adenocarcinoma cell clone HT-29-D4. *J Biol Chem* 1989; **264**: 10282–6.
16. Peehl DM, Wong ST, Stamey TA. Cytostatic effects of suramin on prostate cancer cell cultures from primary tumours. *J Urol* 1991; **145**: 624–30.
17. Spiegelman Z, Dowers A, Kennedy S, *et al.* Antiproliferative effects of suramin on lymphoid cells. *Cancer Res* 1987; **47**: 4694–8.
18. Cheson JE, Levine AM, Mildvan D, *et al.* Suramin therapy in AIDS and related disorders; reports of the US suramin working group. *J Am Med Ass* 1987; **258**: 1347–51.
19. Stein CA, LaRocca RV, Thomas R, *et al.* Suramin: an anticancer drug with a unique mechanism of action. *J Clin Oncol* 1989; **7**: 499–508.
20. Allbriton ML, Verret RC, Walley RC, *et al.* Calcium ion concentration and DNA fragmentation in target cell destruction by cytolytic T lymphocytes. *J Exp Med* 1988; **167**: 514–7.
21. Allen PD, Johnston DH, Macey MG, *et al.* Modulation of CD4 by suramin. *Clin Exp Immunol* 1993; **91**: 141–6.
22. Lopez DM, Lopez-Cepero M, Watson GA, *et al.* Modulation of the immune response by mammary tumour derived factors. *Cancer Invest* 1991; **9**: 643–53.
23. Greenberg PD. Adoptive T cell therapy of tumours: mechanisms operative in the recognition and elimination of tumour cells. *Adv Immunol* 1991; **49**: 281–55.
24. Widmer MB, Acres RB, Sassenfeld HM, *et al.* Regulation of cytolytic cell populations from human peripheral blood by B cell stimulatory factor 1 (interleukin-4). *J Exp Med* 1987; **166**: 1447–55.
25. Balch CM, Dougherty PA, Cloud GA, *et al.* Prostaglandin E_2 mediated suppression of cellular immunity in colon cancer patients. *Surgery* 1984; **95**: 71–7.
26. Smith KA. The interleukin 2 receptor. *Adv Immunol* 1988; **42**: 165–79.
27. Newman R, Scheider C, Sutherland R, *et al.* The transferrin receptor. *Trends Biochem Sci* 1982; **7**: 397–400.
28. Evans RL, Faldetta TJ, Humphreys RE, *et al.* Peripheral blood T cells sensitised to mixed leukocyte culture synthesise and express Ia like antigens. *J Exp Med* 1978; **148**: 1440–5.
29. Schwarting R, Stein H. Activation antigens: cluster report: CD71. In: Knapp W, Dorken B, Gilks WR, *et al. Leucocyte typing IV: white cell differentiation antigens.* Oxford: Oxford University Press 1989: 455–60.
30. Roberts AB, Sporn MB. Transforming growth factor beta. *Adv Cancer Res* 1988; **51**: 107–47.
31. Kehrl JH, Wakefield LM, Roberts AB, *et al.* Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. *J Exp Med* 1986; **163**: 1037–50.
32. Espevik T, Figari IS, Ranges GE, *et al.* Transforming growth factor beta 1 (TGFβ1) and recombinant human tumour necrosis factor alpha reciprocally regulate generation of lymphokine activated killer cell activity. Comparison between natural porcine platelet derived TGFβ

and TGFβ2 and recombinant human TGFβ1. *J Immunol* 1988; **140**: 2312–6.

33. Brooks B, Chapman K, Lawry J, *et al.* Suppression of lymphokine activated killer (LAK) cell induction mediated by interleukin 4 and transforming growth factor beta 1. Effect of addition of exogenous tumour necrosis factor alpha and interferon gamma and measurment of their endogenous production. *Clin Exp Immunol* 1990; **82**: 583–9.
34. Ranges GE, Figari IS, Espevik T, *et al.* Inhibition of cytotoxic T cell development by transforming growth factor β and reversal by tumour necrosis factor α. *J Exp Med* 1987; **166**: 991–8.
35. Mills GB, Zhang N, May C, *et al.* Suramin prevents binding of interleukin 2 to its receptor: a possible mechanism of immunosuppression. *Cancer Res* 1990; **50**: 3036–42.

(Received 24 October 1994; received in revised form 1 December 1994; accepted 15 December 1994)