[Cancer Biology & Therapy 1:6, 626-630, November/December 2002]; ©2002 Landes Bioscience

Exegesis

Ribavirin in Cancer Immunotherapies

Controlling Nitric Oxide Helps Generate Cytotoxic Lymphocyte

R.E. Kast

*Correspondence to: R.E.Kast, M.D.; College of Medicine; University of Vermont; 2 Church St.; Burlington, Vermont 05401 USA; Tel.: 802-863-2462; Email: rekast@email.com

Received 8/21/02; Accepted 10/30/02

Previously published online as a CB&T "Paper in Press" at: http://landesbioscience.com/journals/cbt/

KEY WORDS

Adjuvant, Cancer immunotherapy, Hepatitis C, Inflammation, Interferon-alpha, Lymphocyte, Macrophage, Nitric oxide

ABSTRACT

Either ribavirin, RBV, or cyclophosphamide, CY, can shift an immune response from Th2 towards a Th1 cytokine profile. CY is used in this role in various current cancer immunotherapy attempts but with mixed success. More potent and reliable immunoadjuvants and Th 1 response biasing methods are needed. RBV is used today mainly to augment interferon-alpha treatment of hepatitis C. RBV shifts an immune response from Th2 towards Th1 more effectively than CY and may be a safe and useful adjuvant for current cancer immunotherapeutic efforts. RBV is thought to act by inhibition of tetrahydrobiopterin synthesis. Tetrahydrobiopterin is an essential co-factor for all known isoforms of nitric oxide synthase. Lowered nitric oxide favors Th1 development as high levels favor Th2 weighting.

INTRODUCTION

This paper reviews some aspects of ribavirin, RBV, that are of particular interest to cancer immunologists. It leads to several surprising conclusions. As a help to understanding this story a brief prelude reviewing the use of cyclophosphamide, CY, to increase tissue destructive immune responses will be presented. It will be shown that there are immunological similarities between RBV and CY in their shaping of immune responses.

RBV is a 244 kDa guanosine analogue depicted in Figure 1 that is considered a broad spectrum anti-viral agent with activity against both DNA and RNA viruses. RBV inhibits inosinic acid dehydrogenase,1 and thereby reduces lymphocyte guanosine pools.[1] It is being looked at as having potential immunomodulating effects. RBV's oral availability is good; it's relatively well tolerated with a terminal half-life of 298 hours. Steady state is seen clinically after several weeks of use. The major side effect is hemolytic anemia, although not all of those so affected need stop treatment. Induction of mood lability and depression are occasionally a problem. RBV doubles sustained viral clearance rates to interferon-alpha, IFN-α, treatment of chronic hepatitis C infection, HCV, in humans. Yet RBV treatment as monotherapy doesn't change HCV titers at all.

CY depicted in Figure 2, is a 279 kDa pro-drug alkylating agent used widely in cytotoxic, cytoablative treatment attempts for several cancers. It is thought to work by creating inter-strand cross links in DNA and consequent cell death, particularly in rapidly dividing cells. Used in this way CY is profoundly immunosuppressive to both cell mediated and antibody responses. Given as a single much lower dose than that used in cytoablative cancer treatments, CY can show an immune response enhancing effect. CY is used to enhance the evoked immune response in several past and current experimental cancer immunotherapy attempts.

This paper presents data indicating that as immunization adjuvant, both RBV and CY can under certain circumstances and by different mechanisms, similarly shape an immune response towards one more effective in killing malignant, infected, or otherwise antigenetically altered host cells. The data indicates RBV may be safer and more potent than CY in this regard and should be tried in augmentation of current cancer immunotherapy attempts.

LYMPHOCYTES TH1 AND TH2

$CD4^+$ cells (T helper lymphocytes, Th) interact with dendritic cells (usually monocyte lineage cells) most commonly within the T lymphocyte rich areas of the lymph node. It seems to be here that much of the qualitative direction or proportion determination of Th cells takes place, to become a Th1 or Th2 lymphocyte.[2,3] Th1 lymphocytes secrete interferon-γ, IFN-γ, and Il-2 among others, and Th2 lymphocytes secrete Il-4 and Il-10 among others.

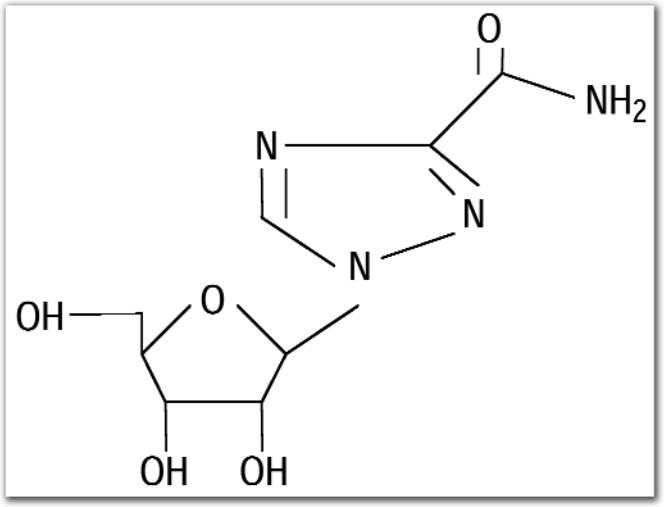

Figure 1. Chemical structure of ribavirin: 244 kDa, terminal half-life in humans, 298 hours.

Cell mediated and soluble antibody responses can be promoted by either Th1 or Th2 cells but cell mediated prominent responses are Th1 associated, Th2 are antibody prominent. Il-2 and IFN-γ promote strong cell mediated, cytotoxic responses, and strong macrophage activation with hundred fold increased NO production and increased other microbiocidal qualities particularly suited to killing of Mycobacteria and intracellular organisms like Listeria monocytogenes (Th1/Th2 reviewed in refs. 2, 3). Virus infected cells', or malignant cells' destruction as aimed for in cancer immunotherapies has been shown to often be Th1 mediated, although not universally so. In addition, elimination of hepatitis C infection and destruction of pancreatic islet beta cells in type I (insulin dependent) diabetes (IDDM) are predominantly Th1 driven. Th2 driven antigen responses tend to be IgG1, IgE, and mast cell and eosinophil prominent responses more suited to elimination of extracellular organisms, soluble circulating foreign proteins, larger antigen loads such as helminths, and other parasites. Th2 development has elements of a default response; though it is actively promoted by cytokines like Il-4, lower antigen-receptor avidity, larger antigen load, and other influences. Immune responses usually involve both Th1 and Th2- the reviewed data and our discussion of CY and RBV will be focused on response weighting to one or the other.

CYCLOPHOSPHAMIDE

Non-obese diabetic, NOD, mice are a strain that spontaneously develop IDDM later in life. They manifest an inflammatory lymphocytic infiltrate in the pancreas islets, insulitis, long before overt IDDM. This pre-diabetic smoldering insulitis is mediated predominantly by Th2 lymphocytes.[4,5] At this stage, there is little or no beta cell loss. The smoldering Th2 prominent insulitis lasts months to a year. As NOD mice age the islet infiltrating lymphocytes become more balanced, Th1 and Th2 and beta cell loss rate increases.[6] At the point of massive loss of beta cells and insulin dependence, a predominantly Th1 infiltrate is seen.[7] Beta cells are exquisitely sensitive to destruction by Th1 cytokines such as Il-1β, TNF-α, and IFN-γ.[8]

A single pulse of low dose CY will accelerate the above transition, ending typically after two weeks post injection with most mice exhibiting full IDDM.[8,9] After the CY pulse, lymphocyte Il-12 gene activation is seen[10] and Il-12 antagonists given post-CY dampen the

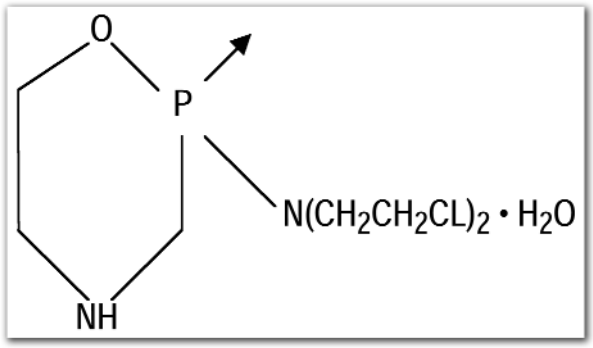

Figure 2. Chemical structure of cyclophosphamide, 279 kDa.

induced insulitis acceleration.[11,12] CY accelerated insulitis, like the slower naturally occurring one, is associated with loss of islet Th2 response, islet beta cell destruction being Th1 mediated.[7,8] Th2 polarizing agents prevent or delay CY acceleration of IDDM in NOD mice, and Th2 cytokines Il-10 and Il-4 are protective to the islets by retarding Th2 to Th1 transition.[8]

The mechanism by which CY accelerates Th2 to Th1 weighting shift in the insulitis of NOD mice is unclear but IFN-γ producers (Th1 lymphocytes) seem to be relatively resistant to CY cytotoxic effects,[13] conferring selective advantage on that lymphocyte subclass. Il-4 is important to Th2 lymphocyte development. It is produced mainly by lymphocytes that have undergone several divisions (reviewed in refs. 2 and 3). By selective killing of actively dividing lymphocytes, the negative selection creates a relative positive selection of Th1 cells.

Th1 weighting shift induced by CY is being used in attempts to increase effectiveness of cancer immunotherapies meeting with varying success in human malignancy and animal models. Single low dose CY has given a weighting shift from Il-10 (Th2) to increased Il-2 and IFN-gamma (Th1) responses in a murine lymphoma model with resultant decreased metastasis.[14] Such pre-immunization low dose CY pulse has also shown benefit in experimental murine models of melanoma,[15] plasmacytoma,[16] fibrosarcoma,[17] and sarcoma.[18] Th1 mediated, macrophage effected elimination of experimental murine tumors can be seen post CY plus Il-12 but with neither used alone.[19] Fourfold peritumoral TNF-alpha and a Th2 to Th1 shift was documented in the fibrosarcoma model when lipopolysaccharide was added to CY pulse.[17]

In human breast and other cancers, low dose CY has shown some immunological and clinical benefit with immunization with a proprietary vaccine (Theratope, an STn-keyhole limpet hemocyanin conjugate given with a proprietary adjuvant Detox).[20,21] Theratope with CY augmentation is currently in two large phase 3 trials for breast and colon cancer. However there have been failures to show benefit of low dose CY: Human trials in breast and colon cancer of MUC-1 conjugated to mannan have shown no clinical or immunological effects of low dose CY,[22] nor was CY of clinical or immunological benefit in a human melanoma vaccine trial.[23] In addition, of concern are observations of pulse CY with methylpresnisolone being used successfully used to induce Th1 to Th2 shift with increased Il-4 in humans.[24]

Conclusions about current clinical use of single low dose CY prior to cancer immunotherapies: CY can in some situations shift an immune response from what would have been Th2 weighted to a Th1 weighted response but there have been clinical failures to

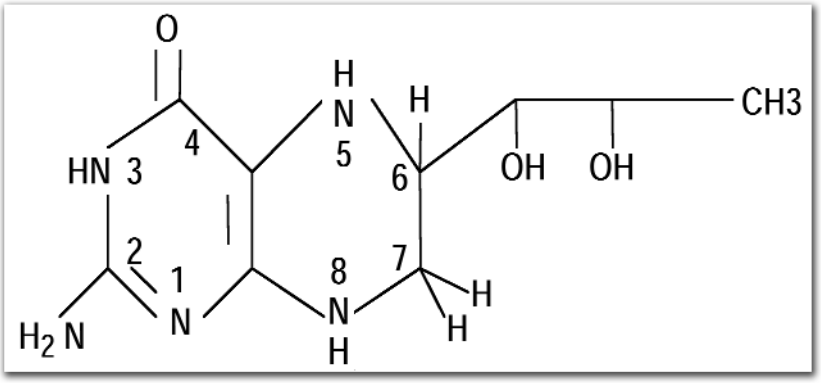

Figure 3. 5, 6, 7, 8, tetrahydrobiopterin, BH4. The pteridine nucleus is numbered in standard fashion.

achieve this. Th1 biasing attribute of pre-vaccination CY can be used with therapeutic cancer immunization attempts to increase tissue destructive immune responses but constructive responses are unreliably achieved. Under some circumstances, depending on dose, timing with respect to antigen etc, CY can be tolerogenic, or Th2 shifting, or generally immunosuppressive, particularly if given after antigen. People with cancer have presumably already been presented with the relevant antigen at the time of our immunization efforts so these threats are real. There is no immunological reason preventing concurrent Th1 biasing by CY and tolerogenizing or immunosuppressive effects all occurring within the same host, the net effect being whichever predominates. Even should Th1 predominate it would be expected to be weakened by the tolerogenic or Th2 biasing component. Better Th1 biasing treatments are needed.

RIBAVIRIN

It is variously estimated that 1 to 3% of humanity is currently chronically infected with hepatitis C virus, HCV, a single stranded positive sense RNA virus of the Flaviviridae family. The replication time is 24 hours and an average total body burden is 1012 copies. About a third of those infected eventually die of cirrhosis or hepatocellular carcinoma because of their infection. Less than 20% of those acutely infected clear the virus, the rest becoming chronically infected for decades, a significant fraction of whom die of HCV related illnesses.[25] HCV infection is also a major cause world wide of disability due to chronic mild encephalopathy, chronic pain, fatigue and malaise.[26]

There are four immunological curiosities about HCV infection, currently unexplained:

1. HCV is not itself hepatocellular toxic. Florid replication can occur for years in livers of near normal histology;
2. The observed steady state HCV numbers over years with a replication time of 24 hours implies viral clearance time of 24 hours. HCV is therefore constantly being cleared and produced. Several HCV antigens are recognized as foreign and both antibody and cell mediated immune responses can be demonstrated to these antigens yet established infection is rarely cleared without treatment;
3. HCV does not generally replicate in hepatocyte cultures in vitro;
4. RBV as monotherapy has no measurable effect on viral numbers in infected patients.[27-31] Liver transaminases are lowered during RBV monotherapy of HCV but usually rise to pre-treatment values when RBV is stopped.[29,31] IFN-alpha monotherapy over a one-year period results in about 20% of patients showing sustained clearance of HCV, though more will have temporary reductions in viral numbers during treatment.[25,32] Addition of RBV (1000 to 1200 mg/day p.o.) to IFN-α raises sustained clearance to 40%.[32]

These four curiosities would be explained if the theory that there is some aspect of the inflammatory, immune, or other host response to HCV or HCV infected hepatocytes that is required for replication were correct.

The current standard theory on how HCV avoids clearance by the immune system is that its high genetic variability, with consequent antigenic drifting results in immune system clearing one antigenic variant as the next comes along (25 for example). That there is high genetic variability and antigenic drift during an HCV infection seems to be proven but there is no evidence that the immune system completely clears any specific HCV subpopulation during a chronic infection, and there is good evidence that the drift does not encompass T cell recognized epitopes.[28] No significant effect of RBV on HCV amino acid sequence evolution was seen.[28]

RBV mediates immune response weighting shift, Th2 towards Th1. How it does so is unclear. Low dose (1 μg per Petri dish) RBV increases in vitro murine antibody response to sheep erythrocytes[33] by suppression of T suppressor subpopulations. In experimental immunization of mice to hepatitis B antigens, 25 times the amounts of Th1 cytokines Il-2 and IFN-γ are seen in mice also receiving RBV.[27] Also noted in this murine study was decreased IgG1 (which is positively regulated by Th2 cells) and increased IgG2 (positive regulated by Th1 cells) after RBV. Th2 to Th1 skewing was observed in a murine hepatitis model both in vivo and in vitro after RBV or its L enantiomer.[34] RBV and its analogues are being explored to bias the immune system from Th2 towards Th1 in human disease treatment.[34,35] Since non-dividing lymphocytes can meet their guanosine requirements largely by salvage but stimulated lymphocytes must meet these requirements by de novo synthesis,[36] Th2 biased lymphocytes would be selected against during RBV treatment since they must pass through several cell divisions and have consequent exogenous guanosine requirements before taking on Th2 qualities.[3]

THE NITRIC OXIDE CONNECTION

NO (.N=O) is a highly reactive free radical synthesized in vessel endothelium, immune cells, brain, and other tissues. NO is a killer. Among the many paths the immune system uses to kill cells, NO mediated cell death figures prominently. Although essential to host defense, at some point of increasing NO, toxicity to host lymphocytes and the NO synthesizing macrophages themselves becomes evident.[37] NO is synthesized by enzymatic cleavage of the guanido nitrogen of arginine by nitric oxide synthase, NOS, that exists in three different isoforms; constitutive, (cNOS or NOS 3), inducible, (iNOS or NOS 2) and neural, (nNOS or NOS 1).[37] TNF-α, Il-1 beta, and immune cell activation generally give rise to increasing amounts of iNOS mRNA followed by several hundred times the NO output of the given cell. All three isoforms of NOS have an absolute requirement for tetrahydrobiopterin, BH4, (Fig. 3) to function catalytically in NO synthesis.[37] BH4 is synthesized in mammalian cells from guanosine 5′ triphosphate, GTP. Since RBV lowers intracellular guanosine pools, BH4 levels drop, and with it the ability to generate NO.[37,38]

iNOS is considerably up-regulated in liver mononuclear cells during chronic infection with HCV.[39-43] This iNOS up-regulation is diffuse, seen evenly throughout the liver even when fibrosis scores

are low and HCV antigen immunostains few isolated and scattered islands of hepatocytes.[43] This is prima facie evidence that HCV is doing something to upregulate NO production beyond its immediate replicative microenvironment. Current IFN-α treatments of HCV actually further up-regulate this already elevated intrahepatic NOS activity[44,45] thereby potentially increasing, as outlined below, an NO mediated negative selection pressure on HCV responding cytotoxic Th1 pattern lymphocytes.

Further evidence for NO mediated negative selection pressure on HCV responding lymphocytes comes from studies on circulating levels of lipopolysaccharide, LPS, a major component of the cell wall of Gram negative bacteria (endotoxin is the historical term for LPS). LPS is one of the most potent inducers of NO synthesis known.[46] HCV patients who go on to durably clear their viremia under IFN-alpha plus RBV treatment have lower pre-treatment LPS levels than those who fail to clear.[47] In addition, 100% of those to clear have end of treatment undetectable levels of LPS while half of those failing to clear still show moderate LPS levels.[47]

The additional 20% of complete sustained responders to RBV-IFN over IFN monotherapy is attributable to RBV's prevention of IFN-α mediated increase of NO in the milieu of cytotoxic lymphocytes. In the 20% of IFN-α monotherapy patients who would have cleared if RBV had been added, excessive NO killed the killers.

A similar situation has been commonly recognized in parasitology and mycology. High levels of NO can be required for parasite killing but high levels of NO also can kill responding host T lymphocytes. At some point of increasing NO, the latter force predominates and the parasite wins the battle. At somewhat lower doses the parasite is killed, the host wins. At lower yet levels of NO the parasite wins again with the failure of NO mediated parasite killing (ref. 48 for paracoccidioidomycosis and ref. 49 for echinococcosis are examples). HCV up-regulates NO synthesis, high NO levels help HCV survive, RBV lowers NO helping the body generate lymphocytes capable of killing HCV infected cells.

The huge increases of NO seen during overwhelming sepsis contribute to the observed immunosuppression of that state.[50] The excessive NO produced by activated macrophages are seen to inhibit antigen driven T lymphocyte proliferation in diverse other situations as well.[50-53] IFN-γ produced by activated Th1 lymphocytes (or in response to therapeutic use of IFN-α) induces several hundred- fold increase in NO production by macrophages.[53] High NO levels are preferentially cytotoxic to antigen responding Th1 lymphocytes compared to Th2 cells.[52] Thus NO, produced by macrophage lineage cells limits expansion of the antigen responding T lymphocyte population. In cancer immunotherapies and in HCV, too soon it seems.

The above discussion of RBV and NO goes a long way toward explaining the immunological curiosities of HCV mentioned earlier.

DISCUSSION

Th1 biased immune responses are often sought in current cancer immunotherapy attempts. They are thought to be more effective in malignant cell killing than Th2 biased responses. Low dose CY is used for such biasing in current trials of cancer vaccines in humans but RBV may be more effective in this role. NO contributes to the development and evolution of all three phases of an immune response-initiation, maintenance, and ending. Excessive or inadequate NO levels can impair host defenses. In pathologically high NO states, RBV, by depriving NOS of BH4, causes NO synthetic ability to fall. The brake on evolution of effective anti-HCV or anti-malignant cell immune responses is thereby lessened. The evidence from RBV use in IFN-α based treatment of HCV indicate RBV may be more effective in this role in cancer immunotherapies than is the currently used CY. A test of this would be checking RBV's ability to accelerate IDDM in NOD mice compared to the well-characterized actions of CY.

RBV could prove useful in other diseases where NO overproduction is pathophysiologically important. For example all multiple myeloma, MM, cells investigated over expressed NOS.[54] MM is a bone eroding malignant expansion of a post germinal center B lymphocyte clone. Current treatments are rarely curative but IFN-α can prolong temporary remission after cytotoxic chemotherapy. If overproduction of NO is part of MM cell's survival strategy as conjectured for HCV, then RBV addition should prolong yet further IFN-α remissions in MM.

References

1. Fairbanks LD, Bofill M, Ruckemann K, Simmonds HA. Importance of ribonucleotide to proliferating T lymphocytes from healthy humans. J Biol Chem 1995; 270: 29682-9
2. Jankovic D, Liu Z, Gause WC. Th1 and Th2 cell commitment during infectious disease. Trends Immunol 2001; 22:450-7
3. Farrar JD, Asnagli H, Murphy KM. T helper subset development: roles of instruction, selection, and transcription. J Clin Invest 2002; 109:431-435
4. Martin S, Hibino T, Faust A, Kleemann R, Kolb H. Differential expression of ICAM-1 and LFA-1 versus L-selectin and VCAM-1 in insulitis of NOD mice and association with both Th-1 and Th2 infiltrates. J Autoimmun 1996; 9:637-43
5. Rothe H, Faust A, Schade U, Kleemann R, Bosse G, Hibino T, Martin S, et al. Cyclophosphamide treatment of female NOD mice causes enhanced iNOS and IFN-gamma, but not of Il-4. Diabetologia 1994; 37:1154-8
6. Faulkner-Jones BE, Dempsey-Collier M, Mandel TE, Harrison LC. Both Th1 and Th2 cytokine mRNA are expressed in NOD mouse pancreas in vivo. Autoimmunity 1996; 23:99-110
7. Nitta Y, Kawamoto S, Tashiro, et al. Il-12 plays a pathologic role at the inflammatory loci in the development of diabetes in NOD mice. J Autoimmun 2001; 16:97-104
8. Reddy S, Karanam M, Poole CA, Ross JM. Dual label immunohistochemical study of Il-4 and IFN-gamma cells within pancreas of NOD mouse during disease acceleration with cyclophosphamide. Autoimmunity 2000; 32:181-92
9. Martin S, van den Engel NK, Vinke A, et al. Dominant role of ICAM-1 in the pathogenesis of autoimmune diabetes in NOD mice. J Autoimmun 2001; 17:109-17
10. Rothe H, Burkart V, Faust A, Kolb H. Il-12 gene expression mediates the accelerating effect of cyclophosphamide in autoimmune disease. Ann NY Acad Sci 1996; 795:397-9
11. Trembleau S, Penna G, Gregori S, Gately MK, Adorini L. Deviation of pancreas infiltrating cells to Th2 by Il-12 antagonist administration inhibits autoimmune diabetes. Eur J Immunol 1997; 27:2330-9
12. Rothe H, O'Hara RM Jr, Martin S, Kolb H. Suppression of cyclophosphamide induced diabetes and pancreatic Th1 reactivity in NOD mice with the Il-12 antagonist Il-12(p40)2. Diabetologia 1997; 40:641-6
13. Ablamunits V, Quintana F, Reshef T, Elias D, Cohen IR. Acceleration of diabetes by cyclophosphamide is associated with an enhanced IFN-gamma secretion pathway. J Autoimmun 1999; 13:383-92
14. Matar P, Rozados VR, Gervasoni SLLI, Scharovsky GO. Th2/Th1 switch induced by a single low dose of cyclophosphamide in a rat metastatic lymphoma model. Cancer Immunol Immunother 2002; 50:588-96
15. Cao X, Zhang W, Wan T, Yu Y, Tao Q, Wang J. Enhanced antitumor immune responses of Il-2 gene modified tumor vaccine by combination with Il-1 and low dose cyclophosphamide. J Exp Clin Cancer Res 1999; 18:173-9.
16. Li L, Okino T, Kan N, et al. Analysis of effector cells in tumor bearing mice pre-treated with active specific immunization followed by cyclophosphamide. Biotherapy 1998; 11:223-8.
17. Inagawa H, Nishizawa T, Honda T, Nakamoto T, Takagi K, Soma G. Mechanisms by which chemotherapeutic agents augment the antitumor effects of TNF-alpha: involvement of the pattern shift of cytokines from Th2 to Th1 in tumor lesions. Anticancer Res 1998; 18(5D):3957-64.
18. Livingston PO, DeLeo AB, Jones M, Oettgen HF. Comparison of approaches for augmenting the serologic response to the individually specific methylcholanthrene induced sarcoma MethA: pretreatment with cyclophosphamide is most effective. J Immunol 1983; 131:2601-5.
19. Tsung K, Meko JB, Tsung YL, Peplinski GR, Norton JA. Immune response against large tumors eradicated by treatment with cyclophosphamide and Il-12. J Immunol 1998; 160:1369-77

20. MacLean GD, Reddish MA, Koganty RR, Longenecker BM. Antibodies against mucin associated sialyl-Tn epitopes correlate with survival of metastatic adenocarcinoma patients undergoing active specific immunotherapy with synthetic STn vaccine. J Immunother Emphasis Tumor Immunol 1996; 19:59-68.
21. MacLean GD, Miles DW, Rubens RD, Reddish MA, Longenecker BM. Enhancing the effect of Theratope STn-KLH cancer vaccine in patients with metastatic breast cancer by pretreatment with low dose i.v. cyclophosphamide. J Immunother Emphasis Tumor Immunol 1996; 19:309-16.
22. Karanikas V, Thynne G, Mitchell P, et al. Mannan mucin-1 peptide immunization: influence of cyclophosphamide and the route of administration. J Immunol 2001; 24:172-83.
23. Euhus DM, Gupta RK, Morton DL. Induction of antibodies to a tumor associated antigen by immunization with a whole melanoma cell vaccine. Cancer Immunol Immunother 1989; 29:247-54.
24. Smith DR, Balashov KE, Hafler DA, Khoury SJ, Weiner HL. Immune deviation following pulse cyclophosphamide methylprednisolone treatment of MS: increased Il-4 and associated eosinophilia. Ann Neurol 1997; 42:313-8.
25. Boyer N, Marcellin P. Pathogenesis, diagnosis and management of hepatitis C. J Hepatol 2000; 32(1 Suppl):98-112.
26. Kast RE. Chronic mild encephalopathy of hepatitis C virus: evidence for a common psychiatric illness. Accepted for publication, Advances in Psychiatric Treatment 2002.
27. Hultgren C, Milich DR, Weiland O, Sallberg M. The antiviral compound ribavirin modulates T1/Th2 subset balance in hepatitis B and C virus specific immune responses. J Gen Virol 1998; 79(Pt 10):2381-91.
28. Querenghi F, Yu Q, Billaud G, Maetens G, Trepo C, Zoulim F. Evolution of hepatitis C virus genome in chronically infected patients receiving ribavirin monotherapy. J Viral Hepatol 2001; 8:120-31.
29. Reichard O, Sonnerborg A, Weiland O. HCV RNA titers prior to, during, and after oral RBV treatment. J Med Virol 1993; 41:99-102.
30. Di Bisceglie AM, Hoofnagle JH, Krawczynski K. Changes in HCV antigen in liver with antiviral therapy. Gastroenterology 1993; 105:858-62.
31. Reichard O, Andersson J, Schvarcz R, Weiland O. RBV treatment for chronic HCV. Lancet 1991; 337:1058-61.
32. McHutchison JG, Shad JA, Gordon SC, et al. Predicting response to initial therapy with IFN plus ribavirin in chronic HCV using serum HCV RNA results during therapy. J Viral Hepat 2001; 8:414-20.
33. Powers CN, Peavy DL, Knight V. Selective inhibition of functional lymphocyte subpopulations by ribavirin. Antimicrob Agents Chemother 1982; 22:108-14.
34. Tam RC, Ramasamy K, Bard J, Pal B, Lim C, Averett DR. Ribavirin analogue ICN 17261 demonstrates reduced toxicity with retention of both immunomodulatory activity and reduction of hepatitis induced serum alanine transferase levels. Antimicrob Agents Chemother 2000; 44:1276-83.
35. Ramasamy KS, Tam RC, Bard J, Averett DR. Monocyclic L nucleosides with type 1 cytokine inducing activity. J Med Chem 2000; 43:1019-28.
36. Fairbanks LD, Bofill M, Ruckemann K, Simmonds HA. Importance of ribonucleotide availability to proliferating T lymphocytes from healthy humans. J Biol Chem 1995; 270:29682-9.
37. Mills CD. Macrophage arginine metabolism to orinthine/urea or NO/citrulline: A life or death issue. Crit Rev Immunol 2001; 21:399-426.
38. Hurshman AR, Marletta MA. Reactions catalysed by the heme domain of iNOS synthase: evidence for the involvement of BH4 in electron transfer. Biochemistry 2002; 41:3439-56.
39. Hokari A, Zeniya M, Esumi H, et al. Detection of serum nitrite and nitrate in primary biliary cirrhosis: Possible role of nitric oxide in bile duct injury. J Gastroenterol Hepatol 2002; 17:308-15.
40. Schweyer S, Mihm S, Radzun HJ, Hartmann H, Fayyazi A. Liver infiltrating T lymphocytes express IFN-gamma and iNOS in HCV infection. Gut 2000; 46:255-9.
41. Mihm S, Fayyazi A, Ramadori G. Hepatic expression of iNOS transcripts in chronic HCV infection: relation to hepatic viral load and liver injury. Hepatology 1997; 26:451-8.
42. Kane JM 3rd, Shears LL 2nd, Hierholzer C, Ambs S, Billiar TR, Posner MC. Chronic HCV infection: induction of hepatic NOS and proposed mechanisms for carcinogenesis. J Surg Res 1997; 69:321-4.
43. Lake-Bakaar G, Sorbi D, Mazzoccoli V. NO and chronic HCV and HIV infections. Dig Dis Sci 2001; 46:1072-6.
44. Sharara AI, Perkins DJ, Misukonis MA, et al. IFN-alpha activation of human blood mononuclear cells in vitro and in vivo for NOS type 2 mRNA and protein expression:possible relationship of induced NOS 2 to the anti-HCV effects of IFN-alpha in vivo. J Exp Med 1997; 186:1495-502.
45. Brassard DL, Grace MJ, Bordens RW. IFN-alpha as an immunotherapeutic protein. J Leukoc Biol 2002; 71:565-81.
46. Gaillard T, Mulsch A, Busse R, Klein H, Decker K. Regulation of NO production by stimulated rat Kupffer cells. Pathobiology 1991; 59:280-3.
47. Caradonna L, Mastronardi ML, Magrone T, et al. Biological and clinical significance of endotoxinemia in the course of HCV infection. Curr Pharm Des 2002; 8:995-1005.
48. Nascimento FR, Calich VL, Rodriguez D, Russo M. Dual role for NO in paracoccidioidomycosis: essential for resistance, but overproduction associated with susceptibility. J Immunol 2002; 168:4593-600.
49. Li WJ, Gottstein B. NO mediated immunosuppression following murine Echinococcus multilocularis infection. Immunology 1999; 97:107-16.
50. Wright KL, Ward SG. Interactions between phosphatidylinositol 3 kinase and NO: explaining the paradox. Mol Cell Biol Res Commun 2000; 4:137-43.
51. Juedes AE, Ruddle NH. Resident and infiltrating CNS APC's regulate the emergence and resolution of EAE. J Immunol 2001; 166:5168-75.
52. Roozendaal R, Vellenga E, de Jong MA, et al. Resistance of activated Th2 cells to NO induced apoptosis is mediated by gamma glutamyltranspeptidase. Int Immunol 2001; 13:519-28.
53. van der Veen RC, Dietlin TA, Pen L, Gray JD, Hofman FM. Antigen presentation to Th1 but not Th2 cells by macrophages results in NO production and inhibition of T cell proliferation: IFN-gamma is essential but insufficient. Cell Immunol 2000; 206:125-35.
54. Mendes RV, Martins AR, de Nucci G, Murad F, Soares FA. Expression of NOS isoforms and nitrotyrosine immunoreactivity by B cell non-Hodgkin's lymphomas and multiple myeloma. Histopathology 2001; 39:172-8.