Cellular Immune Responses Before and During S2 – Complex Immunotherapy in Head and Neck Cancer Patients

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ABSTRACT:

BACKGROUND:

Host immune response especially cell mediated immunity play an important role in the origin and progression of head and neck cancer.

METHODS:

A total of 63 terminal untreatable stage IV head and neck cancer patients were investigated for cellular immune response before and during S2- complex (synthetic -2) immunotherapy from 1992-1994 in Iraqi Medical Collage – Al-Nahrain University. Cellular immunity were investigated including leukocytes, lymphocytes, T and B lymphocytes and T lymphocytes subsets CD4+ , CD8+ and HLA-DR + cells using monoclonal antibodies by indirect immunoflourescense test. Their results were compared with twenty normal apparently healthy individuals.

RESULTS:

The mean total leukocytes and neutrophil numbers were significantly higher in head and neck cancer (HNC) patients while lymphocytes numbers (T and B) were significantly low. Lymphocytes subsets showed significantly decreased in CD4 % and CD8% not affected. CD4/CD8 ratio was lower than normal. HLA-DR + cells were higher in HNC patients. S2-complex administration showed increased in T and B cells on individual basis , increased CD4 , CD8, HLA-DR+ cells and CD4/CD8 ratio.

CONCLUSION:

Immunotherapy acts as an adjuvant to other modalities of treatment without affecting normal human cells. S2-complex induced normalization of peripheral lymphocytes and cause immunorestoration of immunologic function *in vivo*.

KEY WORDS: head and neck cancer, immunotherapy.

INTRODUCTION:

Host immune responses may play an important role in the origin and progression of head and neck cancer. Studies in the early 1970s revealed a depressed cell mediated immune responses to coetaneous antigenic stimulus skin testing [(1)] and impaired reactivity with an early tumor recurrence after surgery [(2)] . Reports indicate that host immune defence mechanisms in human cancer are closely associated with the pathogenesis of the disease [(3)].This concept is supported by the sporadic cases of head and neck sequmaous cell carcinoma developed in young renal transplant patients as well as immune suppressed young individuals[(4)].The main factors causing the deficient cellular immune response in tumor patients are the impairment in the production of cytokines that may be related to quantitative and qualitative alterations in lymphocytes subsets[(5,6)].

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At the present time, the treatment of disseminated cancers relies on chemotherapy and irradiation, both of which have devastating effects on normal non tumor tissues [(7)]. Because the immune response is highly specific, it has long been hoped that tumor-specific immunity may be used to selectively eradicate tumors without injury the patient [(8)]. Immunotherapy remains a major goal of tumor immunologists. The main strategies for cancer immunotherapy aim to provide antitumor effectors (antibodies and T-cells) to patients by stimulating the patient's own antitumor immune responses by using biological response modifiers like cytokines (IL-2), interferon, levamisole and monoclonal antibodies against various tumor antigens[(9)]. S2-complex (synthetic-2) is one of a new immune therapy used in this study. It is a low molecular weight synthetic organometalic complex (Iraqi classification No.6, International classification No.61 k). It is submitted to the insurance of innocence of invention No.2836 in 15/1/1992. It is used in the treatment of advanced stages of head and neck cancer (HNC) patients in Al-Kadhemia teaching hospital/ oncology unit, Iraqi

medical collage –Al-Naharein University [10]. So we studied the cellular immune response before and during S2-complex immunotherapy in head and neck cancer patients.

PATIENTS AND METHODS:

***PATIENTS GROUP**: Sixty three patients with advanced head and neck sequamous cell carcinoma (HNSCC), most of them failed the conventional forms of therapy (surgery, radiotherapy, chemotherapy) and referred to Oncology Unit in Al-Kadhemya Teaching Hospital- Iraqi Medical College /Al-Nahrain University from year 1992 to 1994 for immunotherapy with S2-complex (0.1-0.5 mg/ kg body weight) for five consecutive days and then once at weekly interval for 6 months. Their aged ranged (13-79 ys.) Male to female ratio was 51/12. Most of them were heavy smokers. They had carcinoma of different sites in the head and neck area (larynx, hypopharaynx, parotid and maxillary sinus ...etc.). * **Control group:** Consisted from twenty healthy individuals. Their age ranged (23-73ys.). All of them were apparently in good general conditions, not smokers and did not take any medication.

***METHODS:** Venous blood was collected from two groups and lymphocytes were separated from the blood [11]. T-lymphocytes and B-cells were estimated [12] .Identification of lymphocytes subsets (CD4, CD8 and HLA-DR + T lymphocytes were counted using monoclonal antibodies [13].Lastly, Leukocytes and neutrophils were also determined.

***STATISTICAL ANALYSIS:** Data was expressed as mean +- standard error mean. Student's t-test was used to find the statistical significance.

RESULTS:

The total mean leukocytes and neutrophils numbers were significantly higher in HNC patients while lymphocytes numbers were significantly lower. Administration of S2-complex did not affect short or long term alteration in above parameters (table-1-). Total T and B lymphocytes number were significantly lower than control group and administration of S2-complexinduced increase in the numbers of these cells after 2 weeks and maintain a state of normalization of these parameters (table-1-). Assessment of lymphocytes response on individual patient's basis showed decrease in the number of patients with lower count (table-2-). Lymphocyte subsets showed that CD4% was significantly reduced and CD8% was not significantly different from the control group. CD4/CD8 ratio was slightly higher in control group. S2-complex injection caused a short and long term increase in CD8 and HLA-DR+ cells. The CD4/CD8 ratio showed an increase in its number as compared with control group (Table-3-). CD4 % increased in its level that was after 6 months showed statistically indifferent from control group.

Table 1:Total Leukocytes, Neutrophil, Total Lymphocytes, Total T-Lymphocytes And B-Cells Counts In Head And Neck Cancer Patients Before And During S2-Complex Treatment And In Healthy Controls

Tests done	Control No.=20	Before No.=63	After 2 ws. No.=51	1 m. No.= 45	2ms. No.=32	3ms. No.=24	4ms. No.=22	5ms. No.=15	6ms. No.=15	Mean from 2ws.-6ms. After treatment
Total leukocyte No. /mm3 X-+SEM	7635 +- 530	9457.1 +- 367.04	9332.3 +-1400.07	9488.8 +- 451.4	8718.7 +- 663.1	10004.1 +- 1025.9	9556.04 +- 1065.9	8457.1 +- 847.3	9306.6 +- 971.4	9266.2
p-value	1	1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
Neutrophil % X-+SEM	61.7 +- 2.1	72.3 +- 1.5	70.2 +- 1.8	72.5 +- 1.5	72.09 +- 2.1	71.4 +- 2.4	71.04 +- 2.3	68.2 +- 3.3	71.2 +- 2.5	70.9
p-value	6,5,4,3, 2,8,7	2	3	4	5	6	7	N.S.	8	
Neutrophil No. /mm3 X-+SEM	4844.1 +- 527.01	6837.3 +- 353.01	6783.9 +- 476.6	7107 +- 434.9	6440.09 +- 596.1	7444.08 +- 1023.2	7056.3 +- 979.9	5971.8 +- 781.9	6910.3 +- 930.8	6816.3
p-value	19,20	19	N.S.	20	N.S.	N.S.	N.S.	N.S.	N.S.	
Total lymphocytes No. /mm3 X-+SEM	2361.7 +- 155.2	1849.8 +- 98.8	1922.3 +- 94.2	1860.6 +- 97.06	1898 +- 170.8	1964.04 +- 180.9	1915.05 +- 201.1	1876.8 +- 226.04	1896.2 +- 136.8	1904.7
p-value	9	9	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
Total T-lymphocyte	1180.3 +-	603.2 +-	784.7 +-	764.8 +-	781.07 +-	719.03 +-	755.06 +-	796.4 +-	840.3 +-	777.3

s No. /mm3 X-+SEM	102.5	57.9	47.8	49.4	65.5	88.5	79.2	96.9	75.8	
p-value	14,13, 12,10, 16,15	10	12	13	14	15	16	N.S.	N.S.	
B-lymphocytes No. /mm3 X-+SEM	119.8 +- 12.7	59.1 +- 4.2	72.5 +- 6.04	78.08 +- 6.8	81.3 +- 13.6	78.2 +- 9.5	87.8 +- 9.7	98.1 +- 17.3	88.2 +- 15.2	84.4
p-value	18,17, 11	11	17	18	N.S.	N.S.	N.S.	N.S.	N.S.	

X=mean, SEM=standared error mean, NS= not significant.
19,18,9,8,7,65,3,1=P<0.02, 13,4,2=P<0.005, 20,17,16,15,14,12=P<0.01, 10,11=P>o.oo1
Normal healthy values:
WBC=4.0-11.0X10 ^{3} /mm^3
L%=20-45%
Total lymphocytes count=1500-400/mm^3
N%=40-75%
Total neutrophil count=200-7500/mm^3
Total T-cells=46-70%
Total T cells count =700-2800/mm^3
B-cells=5-20%
B cells number=75-800/mm^3

Table 2: Number and percent of head and neck cancer (HNC) patients with depressed total lymphocytic count, total T and B lymphocyte counts before and during S2-complex treatment.

Number of patients with depressed	before	2ws. After treatment	1m.	2ms.	3ms.	4ms.	5ms.	6ms.
Total lymphocytic count	17/63 26.9%	10/51 19.6%	8/45 17.7%	5/32 15.6%	3/24 12.5%	3/22 13.6%	3/15 20%	1/15 6.6%
T-lymphocytes Number	28/63 44.4%	12/51 23.5%	7/45 15.5%	4/32 12.5%	4/24 16.6%	3/22 13.6%	2/15 13.3%	1/15 6.6%
B-lymphocytes number	41/63 65%	21/51 41.1%	18/45 40%	14/32 43.7%	7/24 29.1%	6/22 27.2%	5/15 33.3%	5/15 33.3%

Table 3: Mean percent of CD4, CD8, CD4/CD8 ratio and HLA-DR (Ia) positive cells in head and neck cancer patients before and during S2-complex treatment and in healthy controls.

Tests done	Control No.=20	Before No.=63	After 2ws. No.=51	1m. No.=45	2ms. No.=32	3ms. No.=24	4ms. No.=22	5ms. No=15	6ms. No.=15
CD4% X+-SEM	44.3 +- 3.7	31.6 +- 2.5	33.7 +- 2.7	32.1 +- 2.7	27.5 +- 2.09	35.2 +- 3.6	42.6 +- 4.07	37.1 +- 3.7	37.8 +- 5.03
P-value	2,1	1	N.S.	N.S.	3,2	N.S.	3	N.S.	N.S.
CD8% X+-SEM	26 +- 5	29.3 +- 1.8	37.9 +- 2.2	41.3 +- 2.9	35.06 +- 2.8	48.2 +- 3.08	48.04 +- 3.4	44.2 +- 4.2	43.8 +- 4.9
P-value	5,4	8,9,7,6, 11,10	6	7	13,12	12,8,4	13,9,5	10	11
CD4/CD8 X+-SEM	1.5 +- 0.06	1.1 +- 0.1	0.9 +- 0.08	0.7 +- 0.05	0.8 +- 0.07	0.7 +- 0.07	0.8 +- 0.07	0.8 +- 0.07	0.8 +- 0.05
P-value	17,16,15, 14,21,20,	24,22, 14,	15	22,16	17	23,18	19	20	24,21

	19,18	23							
HLA-DR Positive cells X+- SEM	16 +- 5	27.6 +- 2.04	35.8 +- 2.2	37.8 +- 2.5	33.4 +- 2.9	44.9 +- 2.8	44.9 +- 3.3	37 +- 3.07	44.1 +- 5.1
P-value	27,26,25, 30,29,28, 31	34,33,32, 35	26	32,28	36,25	36,33,30	34,31	27	35,29

36,35,32,25,24,13,12,11,6,1=P<0.02
27,23,14,10,7,5,3=P<0.01
29,28,26,22,4,2=P<0.005
33,31,30,21,20,19,18,17,16,15,9,8=P>0.001
34=P<0.001

Normal values:

CD4+ = 44 +- 6
CD8+ = 26 +- 5
CD4/CD8 = 1.6 +- 0.7
HLA-DR(Ia)+ cells = 16 +-5

DISSCUSION:

It has often been speculated that host immune defense mechanisms were important in the development and progression of human malignancies. Current evidences suggest that human malignancies contain specific antigens and that the major part of the host's response against tumor. Our study showed impairment in cell mediated immunity in HNC patients. This was in agreement with other reports [2, 14, 15, 16, 17, 18, 19] involving delayed hypersensitivity skin response, T-lymphocytes level and lymphocytes reactivity to mitogens. Although various immune responses can be generated to tumor cells, the response was not sufficient to prevent tumor growth. One approach to cancer treatment is to augment or supplement these natural defense mechanisms[20,21].This study demonstrate that administration of immunotherapy S2-comlex to the HNC patients , induced normalization of the peripheral total T and B cells, their values remained significantly lower than those of the age-matched controls after short and long-term of treatment. Individual patients based analysis indicated that small number of patients remained with decreased counts of T and B cells. Another finding was slightly decreased CD4/CD8 ratio, significant decrease in CD4, increased in CD8 and HLA-DR+ cells. Our results were in agreement with other studies [22, 23, and 24]. After administration of S2-complex, it was able to correct the altered T, B cells and CD4% cells. It was lead to the inversion of CD4/CD8 ratio and significant stimulation of CD8 and HLA-DR+ cells. *In vitro*, studies showed that S2-complex stimulated lymphocytes and macrophages [25]. These results showed that S2-complex caused immunorestoration of immunologic function *in vivo* too. The effects of S2 –complex in enhancement of CD4, CD8, HLA-DR+ cells and inversion of CD4/CD8 ratio due to high level of CD8 cells over those of CD4 subsets. These results provide a new insight for the mechanisms of S2-complex immunomodulating activities in HNC patients.

CONCLUSION:

Immunotherapy acts as an adjuvant to other modalities of treatment without affecting normal human cells. S2-complex induced normalization of peripheral lymphocytes and cause immunorestoration of immunologic function *in vivo*.

RECOMMENDATION:

S2-complex can be considered as one method for immunotherapy and as an adjuvant with other types of HNC treatment.

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