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# Immunomodulatory Serum Components in Head and Neck Cancer

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## INTRODUCTION

IT IS well known that there is a generalised depression of cellular immunity in some individuals with cancer [1, 2]. A large number of serum proteins that have immunosuppressive activity have been found in those cancer patients [3], which may explain this important immunological phenomenon. Among these proteins, an elevation of the serum level of alpha 1-acid glycoprotein (α1-AG) has been reported in patients with malignancies [4]. Immunosuppressive acidic protein (IAP) [5], which is a kind of AG, was purified from the ascitic fluid of stomach cancer patients and has been found to be elevated in the sera of patients with colorectal cancer, ovarian cancer, and similar diseases. Immunosuppressive substance (IS) [6] was identified in the ascitic fluid of a colon cancer patient with gynaecological malignancies. It is also of particular interest that soluble immune complexes (SIC) have frequently been found in the sera of patients with various types of malignant tumours [7–9], and the SIC have been reported as one of the major blocking agents in cell-mediated cytotoxicity to tumour cells in cancer patients [10, 11].

Despite the relative prominence of head and neck cancer, the information on serum components which may contribute the immunomodulation in cancer patients is rather sparse. In this review I introduce such immunomodulatory serum components in patients with head and neck cancer based on our previous works [12, 13].

## IMMUNOSUPPRESSIVE ACIDIC PROTEIN (IAP) AND IMMUNOSUPPRESSIVE SUBSTANCE (IS)

Using analytical isoelectric focusing in polyacrylamide gel at pH 2.5–5, Tamura *et al.* [5] reported the presence of certain serum acidic protein which could be found in large quantities in the sera of cancer patients, but in only small amounts in the sera of healthy persons. This acidic protein was called IAP, because this protein was found to suppress both the phytohaemagglutinin (PHA)-induced lymphocyte blast formation and mixed lymphocyte reaction *in vitro*. Fujii *et al.* [6] extracted an immunosuppressive glycoprotein from the ascitic fluid of a patient with advanced colon cancer. This material was named IS because it suppressed both PHA-induced human lymphocyte blastogenesis *in vitro* and delayed foot pad reaction in mice. It was reported that IS had a molecular weight of approximately 52 000, a sedimentation coefficient of 4.0 S, and showed physicochemical characteristics similar to IAP. A gel isoelectric focusing of IS, however, revealed it to be different from IAP; i.e. it had several bands at pH 2.7–3.3, indicating the structural variety of the sugar moiety.

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*Estimation of IAP and IS in the sera*

IAP and IS in the sera of patients and healthy control are measured by single radial immunodiffusion. In brief, 1.5% agar gel containing rabbit polyclonal IS antiserum diluted to concentrations ranging from 5 to 20% with Veronal Buffer, pH 8.6 (Kureha Chemical Industry, Tokyo, Japan) or rabbit polyclonal IAP antiserum is prepared on a glass or a plastic plate; wells 2.5 mm in diameter were punched out. The commercial plates for the IAP analysis are supplied by Kayaku Antibiotics Research (Tokyo, Japan). Five microlitre aliquots of undiluted sera are applied to each well after incubation for 48 h at 37°C in a wet chamber. The value of IS or IAP is calculated with a calibration curve against purified IS or purified IAP.

*Serum immunosuppressive acidic protein (IAP) in patients with head and neck cancer*

The serum IAP levels and IAP positive rates in 108 sera of head and neck cancer patients and 19 sera of age-matched healthy controls are summarised in Figs 1 and 2. The value of mean + 2 S.D. of controls was used as the cut-off point. The mean IAP levels in the sera of patients with laryngeal carcinoma, maxillary carcinoma, and nasopharyngeal carcinoma were significantly higher than those of the normal controls ($P < 0.05$). The overall positive rate of serum IAP in our series was 37%. As shown in Fig. 1, the positive rate of serum IAP in patients with laryngeal carcinoma was high in the advanced stages.

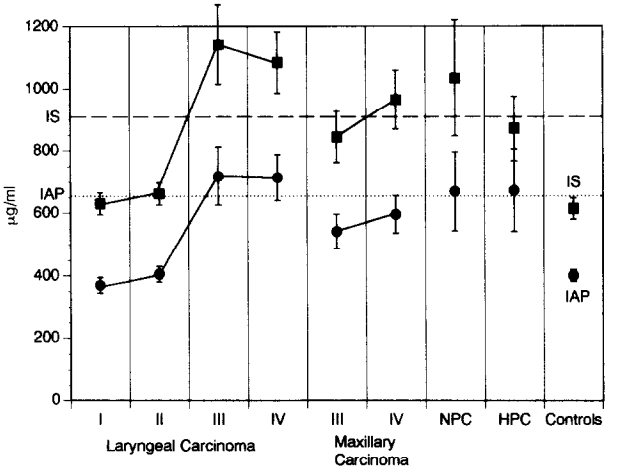


**Fig. 1. Serum levels of immunosuppressive acidic protein (IAP) and immunosuppressive substance (IS) in head and neck cancer patients ($n = 108$) and normal controls ($n = 19$) [12]. The large dotted line at 912 μg/ml and the small dotted line at 658 μg/ml indicate the cut-off points (mean + 2 S.D.) of serum IS and IAP levels, respectively. NPC = nasopharyngeal carcinoma; HPC = hypopharyngeal carcinoma.**

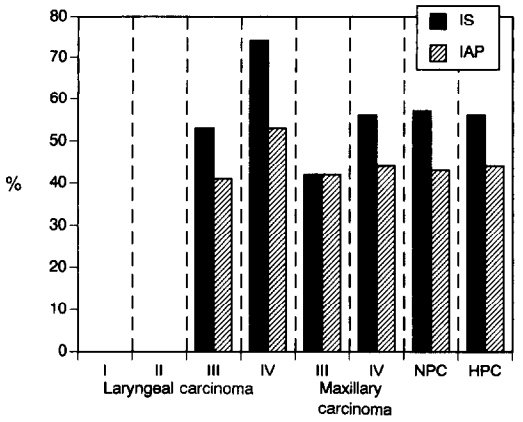


**Fig. 2. The positive rate of serum IS and IAP levels in head and neck cancer patients [12]. Notice the correlation between their positive rates and the disease stage in patients with laryngeal carcinoma. NPC = nasopharyngeal carcinoma; HPC = hypopharyngeal carcinoma.**

*Serum immunosuppressive substance (IS) in patients with head and neck cancer*

Figures 1 and 2 summarise the preoperative level of IS in 108 serum samples from patients and the level of IS from 19 normal controls as analysed by single radial immunodiffusion methods. An IS level over mean + 2 S.D. of normal control was considered as positive. The mean IS levels in the sera of patients with laryngeal carcinoma, maxillary carcinoma, nasopharyngeal carcinoma, and hypopharyngeal carcinoma were significantly higher than those of the normal controls ($P < 0.01$). Patients with stages III and IV laryngeal carcinoma had serum levels that were significantly higher than those with stages I and II ($P < 0.01$). Forty-six per cent of all patients, which is much higher than that of IAP, had positive serum IS levels, and the positive rate in patients with laryngeal carcinoma was found to increase as the disease stage advanced.

*Clinical implications of IAP and IS in head and neck cancer*

Cellular immunity is known to be reduced in cancer patients. The mechanism of such an immunological aberration is not clear; it may be caused by substances produced or induced by cancer cells. Some studies [3, 14, 15] have demonstrated the presence in the serum of patients with advanced cancer of substances which non-specifically inhibit *in vitro* and *in vivo* lymphocyte function. IAP and IS are major factors reported as serum components which show immunoinhibitory function. In animal experiments [16], IS is reported to have a tumour growth promoting activity; i.e. the administration of IS to tumour-transplanted rats caused the tumour to grow and shortened the animals' survival time.

Approximately 40–50% of patients with head and neck cancer had significantly higher serum levels of IAP and IS than were found in the sera of normal controls. Patients with laryngeal carcinoma were shown to have elevated IAP and IS levels according to the advancement of the disease stage. High levels of IAP and IS were frequently found in patients with progressively growing tumours, i.e. in patients in the advanced disease stage and with recurrent disease after the treatment. These results suggest that IAP and IS may reflect tumour burden.

To determine if the treatment against the tumour is the major factor influencing the magnitude of serum IAP and IS measurements, the effect of the treatment on IS and IAP measurements should be evaluated. By serial IS determination, serum IS levels seem to reflect the clinical course of cancer patients. It is interesting that serum IS levels had a tendency to temporarily ascend beyond a normal range during and/or immediately after radiotherapy or surgery. Yamashita *et al.* [17] reported that positive IS levels were detected at a significantly increased frequency in gynaecological malignancies in addition to severe inflammatory diseases rather than in healthy controls or in patients with benign tumours. Considering this result, factors that induce the temporary elevation of serum IS levels might include rapid tumour destruction and/or inflammation caused by the treatment. There was a propensity for serum IS levels to decrease after treatment. However, the levels went up again in the sera of patients with recurrent disease. It is notable that some of the patients showed an elevated serum IS level before the recurrence was clinically evident. This observation was confirmed by the result that serum IS values in patients with recurrent disease were significantly higher than in those patients who had remained free of disease for the 20-month follow-up period ($P < 0.001$). In addition, patients who showed an extremely high level of serum IS (over 1500 μg/ml at admission) died earlier. High IS levels (over 1000 μg/ml) were less frequently seen in patients who had remained free of disease in the follow-up period.

The mechanism of IAP and IS production in tumour-bearing patients is not yet clearly understood. However, it is likely that IAP and IS represent products of the host response to the tumour, and that tumour growth may be the major factor influencing the serum IAP and IS levels. In comparing the clinical usefulness of IAP and IS, the positive rates of IS in the sera of patients with laryngeal carcinoma (stages III and IV), maxillary carcinoma, nasopharyngeal carcinoma, and hypopharyngeal carcinoma were higher than those of IAP. Moreover, the elevation of serum IS levels in advanced laryngeal carcinoma, maxillary carcinoma, and nasopharyngeal carcinoma were statistically more significant than that of IAP when compared with levels in the control group (IS, $P < 0.01$; IAP, $P < 0.05$). In addition, there was no significant difference between the serum IAP level in the patient with hypopharyngeal carcinoma and that in control patients. These facts indicate that the serum level is clinically more useful than the serum IAP level. Considering the data from this study, serum levels of IAP and IS can conceivably be useful parameters for monitoring the clinical course of head and neck cancer patients.

## SOLUBLE IMMUNE COMPLEXES (SIC) IN CANCER PATIENTS

Malignant cell transformation, which has no normal biological control, would very likely result in the appearance of tumour antigenic determinants, which could evoke both humoral and cellular immune responses in cancer patients. It has been suggested that the SIC or free tumour antigens may have a role in modulating cell-mediated immune responses against the tumours.

*Estimation of SIC by solid-phase anti-C3 enzyme linked immunosorbent assay (ELISA)*

This assay method is originated by Pereira *et al.* [18] as a radioimmunoassay and is based on the observation that activation of the complement system by antigen–antibody

complexes leads to the binding of C3 to the complexes. We modified this technique into an enzyme immunoassay (EIA) to preclude the use of radio-isotopes. This anti-C3 assay has some advantages over the Raji cell assay and the C1q assay. For example, it is not affected by non-IC substances such as polyanions, endo-toxins, nucleic acid, rheumatoid factor (RF), and anti-lymphocyte antibodies that are known to interfere with many of the existing IC detection techniques. It detects ICs that fix complement activated not only by way of the classical pathway but also the alternative pathway. Moreover, since anti-Ig antibody is used as the final reagent, this assay permits the detection only of Ig-containing substances. The procedures of solid-phase anti-C3 ELISA for IC in sera have been previously described [13].

*Serum SIC levels in patients with head and neck cancer*

SIC levels in 85 serum samples from preoperative head and neck cancer patients and 20 samples from age-matched healthy controls are summarised in Fig. 3. The SIC level more than mean + 2 S.D. of healthy controls was considered to be positive. In laryngeal carcinoma the level of SIC tended to elevate along with the advancement of the disease stage. The positive rate of SIC in all cancer patients was 29.4%. In all head and neck cancers except hypopharyngeal carcinoma, the positive rate tended to rise with the stage of the disease (Fig. 3).

To evaluate changes in levels of SIC after treatment, the pretreatment and post-treatment SIC levels were compared in 9 patients with laryngeal carcinoma or maxillary carcinoma (the duration of follow-up ranged from 6 to 15 months) (Fig. 4). 3 of 4 patients who had recurrence after the treatment exhibited unchanged or elevated SIC levels, whereas all five patients who showed no evidence of disease exhibited a marked decline of SIC levels below the cut-off value in the sera.

*Clinical implications of SIC in head and neck cancer*

The evidence for the presence of circulating immune complexes in malignancy has been reported by several investigators with different techniques. Theofilopoulos *et al.* [7] used Raji cell assay and reported that 41.6% of patients with malignancy showed positive SIC in the sera. Rossen *et al.* [8] reported a high positive rate (82.8%) of SIC in cancer patients. However, reports referring to the clinical correlation

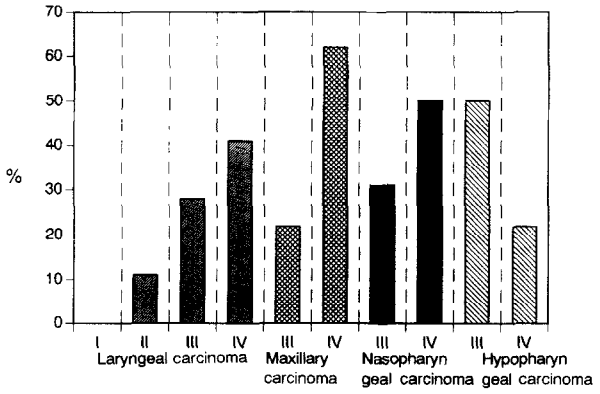


**Fig. 3. Positive rates of soluble imune complexes in the sera of head and neck cancer patients [13]. In all head and neck cancers except hypopharyngeal carcinoma, the positive rate tended to rise with the stage of the disease.**

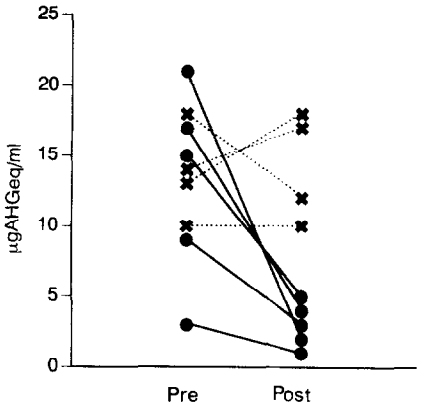


**Fig. 4. Changes in levels of soluble immune complexes (SIC) between pretreatment and posttreatment [13]. Time frames involved were from 6 months to 15 months. ●——●: SIC level of patient who showed no evidence of disease after the treatment; ×- - -×: SIC level of patient who showed recurrence after the treatment.**

of SIC levels in the sera of head and neck cancer patients are rather sparse. Teshima *et al.* [9] documented 50 head and neck cancer patients whose sera exhibited a higher mean value of SIC [16 μg antihaemolytic globulin (AHG) eq/ml] when compared with that of healthy control sera (4.5 μg AHG eq/ml). SICs have been estimated in the sera of oral cancer patients by Scully *et al.* [19] earlier, wherein they have demonstrated increased levels in SICs in untreated patients, which were compared in both malignant and premalignant conditions. Balaram *et al.* [20] also reported elevated SIC levels in patients with oral cancer. They also stressed that the SIC levels in the patients with premalignant lesions were elevated almost to same levels as in the oral cancer patients. By using C1q binding assay, Mukhopadhyaya *et al.* [21] compared SICs levels in the sera of untreated oral cancer patients and those treated with radiation or surgery. Levels of SICs were elevated in 70.8% patients before treatment when compared to healthy controls (4.2% positive). Treated patients showing no evidence of the disease had reduced SIC levels (11.7%), whereas those showing recurrence of the disease had much higher SIC levels (92.3%).

In our series of head and neck cancer, the overall positive rate was 29.4%, which was relatively low compared with previous reports. This is probably due to the different selection of diseases. Rossen *et al.* [8] reported quite high positive rates in cancer patients. However, their population was highly selective, including a large number of patients who were likely to develop recurrences or who had progressively growing neoplasms. In our series high serum SIC were found most frequently in patients with advanced lesions, and positive rate of SICs tended to increase along with the stage of the disease. These data suggest that there may be a relationship between tumour burden and serum SIC in head and neck cancer. Serial studies of serum SIC levels in a limited number of patients indicated that high levels of SIC in the post-treatment cancer patients might suggest the possibility of recurrence.

In cancer patients, however, there may be other ways that SIC are formed in serum, that is, SIC formed by normal tissue antigens released from normal adjunct tissues destroyed by tumour cell growth, or SICs formed by bacterial or viral antigens infected in tumour-bearing hosts who would be vulnerable to infections. Therefore, tests that recognise an antibody

complex associated with specific antigens will be required to determine the specificity of SIC in cancer patients.

## CONCLUSION

Further studies are required to clarify the origin of IAP, IS and SIC, however, it is acceptable that the existence of immunosuppressive factors in sera of head and neck cancer patients may cause the degradation of cellular immunity of host. This fact raises the possibility that removal of immunosuppressive factors from sera of cancer patients may be a feasible immunotherapy for head and neck cancer. From the clinical point of view, it is conceivable that serum IAP, IS and SIC levels are useful parameters for monitoring the disease stage of head and neck cancer patients.


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