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## IMMUNOGLOBULINS IN CARCINOMA OF THE UTERINE CERVIX

by

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In recent years the problem of immunologic competence in patients with solid tumours has attracted much attention. Investigations using different microbial antigens permit the conclusion that in patients with solid tumours the delayed hypersensitivity is impaired (LAMB et coll. 1962, SOLOWEY & RAPAPORT 1965, GROSS 1965, KRANT et coll. 1968). Similar results have been obtained using cross-reacting chemicals like 2,4-dinitrochlorobenzene (LEVIN et coll. 1964, KRANT et coll. 1968).

In contrast to the frequent decrease in the cell mediated immunity, the ability of patients with malignant tumours to respond to antigenic stimuli leading to the formation of humoral antibodies seems to be relatively well preserved. A reduced response to primary immunization with tetanus toxoid has been reported (LYTTON et coll. 1964) but other authors, using different microbial antigens, have observed a relatively well preserved antibody formation capacity in patients with malignant disease (BALCH 1950, PARFENTIJEV et coll. 1951, LARSON & TOMLINSON 1953, SOUTHAM & MOORE 1954, LESKOWITZ et coll. 1957, LEVIN

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et coll. 1964). Additional information on the immunologic profile in patients with malignant diseases could be obtained by measuring serum immunoglobulin concentrations. Changes in IgG and IgM immunoglobulin levels have been observed in the serum of patients with hepatomas (MCKELVEY & FAHEY 1965), Burkitt's lymphoma (NGU et coll. 1966) and lymphogranulomatosis maligna (GOLDMAN & HOBBS 1967). In a series of patients with bronchogenic carcinoma, moderate increases in IgA levels have been observed only in cases with long survival (KRANT et coll. 1968). Recently it has been found that antibodies cytotoxic to malignant melanoma cells are located largely in the IgM fraction of the patients' sera (LEWIS et coll. 1969).

The present investigation is concerned with the serum IgG, IgA and IgM immunoglobulin levels in patients with carcinoma of the uterine cervix. The patients were divided into three groups according to the TNM classification. The immunoglobulin levels were simultaneously evaluated in a group of healthy controls. The determination of serum immunoglobulin levels was done by the radial immunodiffusion method.

*Material.* Seventy-seven consecutive cases of histologically verified squamous cell carcinoma of the uterine cervix were examined. Only previously untreated cases at first admission were included in the study. No patients presenting symptoms or signs of hepatic or renal involvement, or with known distant metastases were included in this investigation. None of the cases had a monoclonal gammopathy.

The patients ranged in age from 30 to 75 years, the arithmetic mean for the group was 54 years (1 SD = 11.8 years).

The extent of the disease according to TNM classification is defined as follows (LEISSNER & NYSTRÖM 1968):

Stage I — Tumour infiltration confined to the cervix with subdivision to the microscopically observed lesion with no palpable mass of tumour.

Stage II — Tumour infiltration extending outside the cervix towards the vagina in the caudal direction or towards the parametrium in the lateral direction.

Stage III — Tumour extent exceeding the caudal third of the vagina or reaching to the pelvic wall.

In the stage I group 24 cases were included, the mean age for the group was 48 years; in the stage II group, 31 cases, mean age 55 years; in the stage III group, 22 cases, mean age 59 years. To the 36 to 70 year age group, which corresponds to the age interval of the two groups of elderly healthy controls reported by JOHANSSON (1968), belonged 61 out of the 77 patients. Considering the 53 patients in stages II and III together, 8 patients did not belong to the age group mentioned above. The control group consisted of 38 female blood donors,

of ages ranging from 21 to 63 years. The arithmetic mean for the control group was 36 years (1 SD = 11.7 years). Out of the 38 women, 18 were in the 36 to 70 year age group.

*Methods.* Blood samples were collected when the extent of the disease was graded. The serum was separated from blood obtained without stasis from the antecubital vein. Aliquots were frozen at  $-20^{\circ}\text{C}$  until determination was performed at a later date.

Quantitative determination of IgG, IgA and IgM immunoglobulins was carried out by the radial immunodiffusion method (MANCINI et coll. 1965, MCKELVEY & FAHEY 1965), which had been slightly modified. All sera were investigated using this same method at the same laboratory. Equal amounts of 2% agar ('Special-Agar Noble, Difco') in phosphate buffer, pH 8.6, and of antisera diluted to a concentration of 1:15 for IgG immunoglobulin and 1:30 for IgA and IgM immunoglobulin, were mixed. The antiserum-agar mixture was poured onto microscopic slides (25 mm  $\times$  75 mm for the determination of IgA and IgM immunoglobulins, and 35 mm  $\times$  75 mm for the determination of IgG immunoglobulin), to form layers 1 mm thick. Using a gel puncher (LKB, Gel puncher, No. 6807 A) two wells, 2.3 mm in diameter, were cut on each slide shortly before use, and filled with 2  $\mu\text{l}$  undiluted serum. After incubation for 72 hours in a humidity chamber at room temperature, the slides were removed and dried overnight. Amido-black was used for staining. The area of precipitation was determined by measuring four diameters at an angle of  $45^{\circ}$ , whereupon the immunoglobulin concentrations were determined by comparing the surface area with the graphic curve plotted for the standard reference sera.

The reference sample was obtained by mixing the serum samples of 28 healthy controls and subsequently preparing aliquots in four dilutions. These dilutions were used for plotting the reference curves for each group of slides. The absolute concentrations of IgG, IgA and IgM immunoglobulins in the reference sample were separately determined using a stabilized standard human-serum (Batch No. 369 A) from Behringwerke AG (Marburg/Lahn, Germany). For comparison a set of reference sera from Hyland Immunoplates at three different concentrations (Hyland Laboratories, Calif., USA), was used. The results with the different standards were fairly similar, and it was decided to accept as absolute values of the reference sample: 932 mg IgG/100 ml, 215 mg IgA/100 ml, and 104 mg IgM/100 ml.

The statistical analysis was made by determining the arithmetic mean and standard error of the mean. Using the t-test, the significance of differences in the immunoglobulin levels was calculated by comparing first, immunoglobulin levels of each stage with the level of the control group and, second, the value of the immunoglobulin level of one stage with that of each of the other two stages.

**Table 1***Comparison of immunoglobulin levels in different Swedish series of healthy women (mg/100 ml)*

	IgG	IgA	IgM
NORBERG (1967)	Mean value $\pm$ SD	Mean value $\pm$ SD	Mean value $\pm$ SD
Elderly women	1 055 $\pm$ 210	201 $\pm$ 78	76 $\pm$ 26
Blood donors women)	1 099 $\pm$ 217	184 $\pm$ 63	77 $\pm$ 30
JOHANSSON et coll. (1968)	Arithmetic mean (range)	Arithmetic mean (range)	Arithmetic mean (range)
20—35 years women	1 267 (756—1 688)	126 (82.5—185)	111 (54.3—159)
36—50 years women	1 454 (961—2 037)	170 (101—264)	108 (37.0—272)
51—70 years women	1 355 (910—1 961)	151 (89.1—212)	86.3 (31.1—160)
Present series	Arithmetic mean $\pm$ SE (range)	Arithmetic mean $\pm$ SE (range)	Arithmetic mean $\pm$ SE (range)
Blood donors women 21—63 years	1 035 $\pm$ 26.6 (719—1 441)	175 $\pm$ 9.1 (93—295)	103 $\pm$ 4.8 (69—167)

## Results

In Table 1 the values of immunoglobulin levels in the control series are compared with data published for the Swedish population by other authors (NORBERG 1967, JOHANSSON et coll. 1968). The mean concentration of IgG immunoglobulin in the sera investigated by the present author is close to that reported by NORBERG and somewhat lower than that presented by JOHANSSON. The IgA levels for the given group determined by the three authors do not show any large differences. In the present series of patients the values of IgM immunoglobulin are higher than those reported by NORBERG but close to the values of JOHANSSON. In the series presented by the latter author the immunoglobulin concentrations for the same sex vary in different age groups. NORBERG found no differences when values for IgG and IgM immunoglobulin concentrations of female blood donors were compared with the group of elderly women.

In Table 2 the mean concentrations for the three classes of immunoglobulins in patients with uterine cervix carcinoma are presented. The frequency distribution of serum immunoglobulin levels is illustrated in Figs 1 and 2. The stage I group value for IgG immunoglobulins was significantly higher than the control group value ( $p < 0.01$ ). In the stage II group the mean concentration of IgG immunoglobulin was lower, the stage III group levels were almost identical with those of the control group. The value of IgG immunoglobulin in stage I was

**Table 2**

*Immunoglobulin levels (mg/100 ml) in cases of uterine cervix carcinoma divided according to TNM classification*

TNM	No.	Mean age (years)	IgG	IgA	IgM
			Arithmetic mean $\pm$ SE range	Arithmetic mean $\pm$ SE range	Arithmetic mean $\pm$ SE range
Stage I	24	48	1 456 $\pm$ 32.2 1 102—1 827	192 $\pm$ 8.6 67—280	111 $\pm$ 5.8 54—201
Stage II	31	55	1 256 $\pm$ 43.1 788—2 149	192 $\pm$ 9.2 90—315	97 $\pm$ 5.2 45—166
Stage III	22	59	1 030 $\pm$ 23.5 700—1 270	203 $\pm$ 6.8 120—295	73 $\pm$ 5.8 27—149
Controls	38	36	1 035 $\pm$ 26.5 719—1 441	175 $\pm$ 9.1 93—295	103 $\pm$ 4.8 69—167

also significantly higher than that in the stage III group ( $p < 0.01$ ). In all three stage groups the value of IgA tended to be higher than in the control group; these differences were not significant, however. No significant differences were observed when the mean values for all three stages were compared with the control value of IgA immunoglobulin. There was a significant difference between the mean concentration of IgM immunoglobulin for the stage I and II groups as compared with the concentration of the control group. The value for the stage III group was significantly lower than that for the control group ( $p < 0.01$ ) and that for the stage I group ( $p < 0.01$ ).

### Discussion

A comparison of the age distribution of the patient group (30 to 75 years, mean age 54 years) with that of the control group (21 to 63 years, mean age 36 years) reveals a considerably greater proportion of younger women in the control group. The question arises whether the differences in immunoglobulin levels noted in the various groups analysed in this paper might simply represent an age dependent variation. As noted above, the majority of stage II and III patients fall into the 36 to 70 year age group. This correlates well with the age distribution in two groups of elderly women presented by JOHANSSON (Table 1). In comparing IgG immunoglobulin levels, there is agreement between our controls and those reported by NORBERG. On the other hand, JOHANSSON's values are

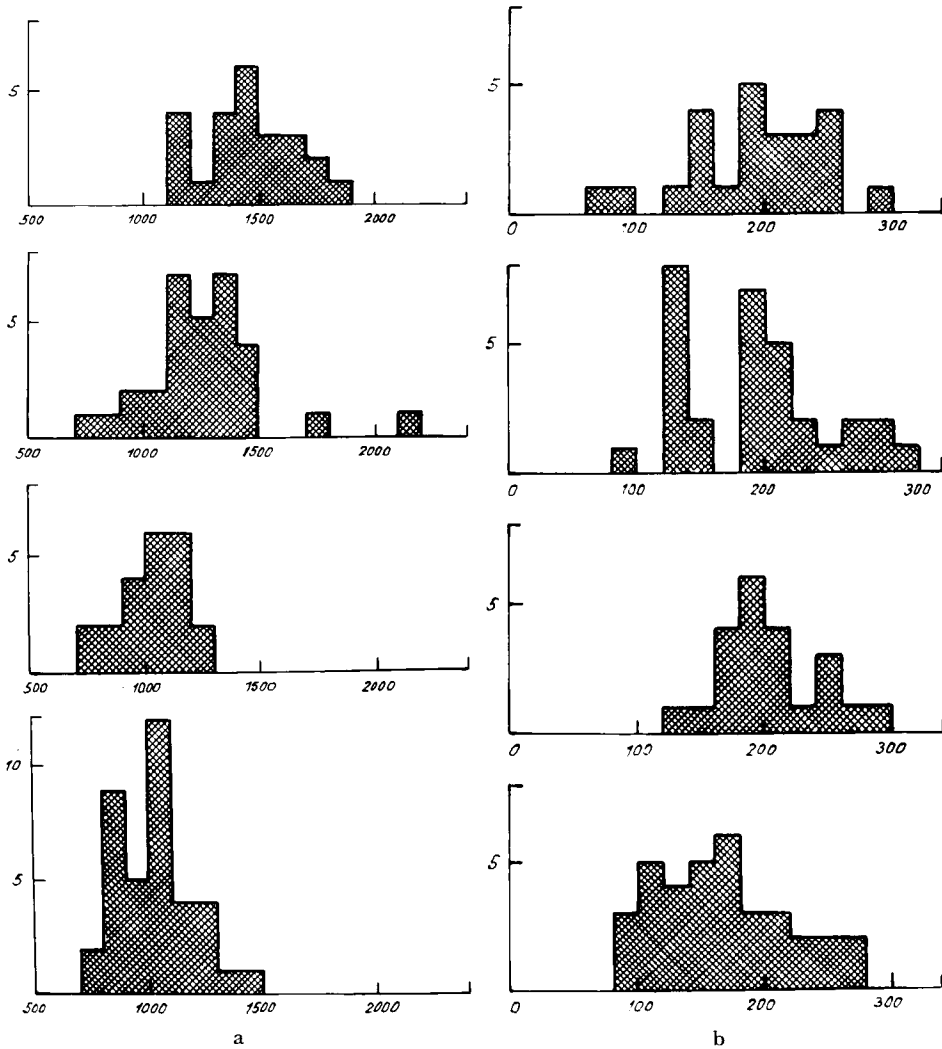


Fig. 1. Frequency distribution of serum IgG (a) and IgA (b) immunoglobulin levels in 77 patients with carcinoma of the uterine cervix compared with 38 healthy controls. From top to bottom: Stage I (34 cases), Stage II (31 cases), Stage III (22 cases) and controls. Values expressed in mg per 100 ml.

higher than both the values in NORBERG's series and the series of controls in this report.

Nonetheless, no consistent age dependent differences could be found in either of the series of these authors. It seems reasonable to conclude that variations in

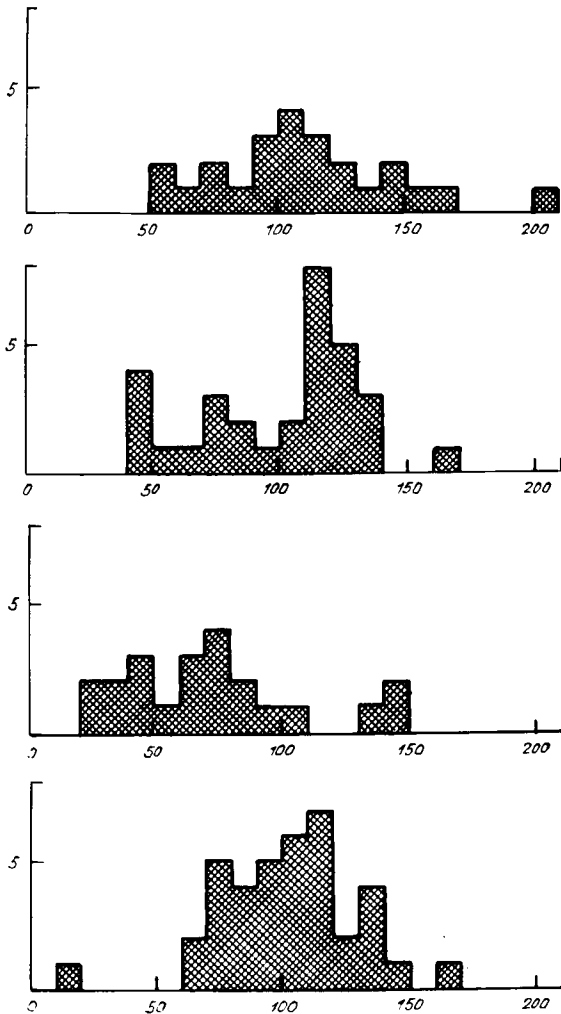


Fig. 2. Frequency distribution of serum IgM immunoglobulin levels in 77 patients with carcinoma of the uterine cervix compared with 38 healthy controls. From top to bottom: Stage I (34 cases), Stage II (31 cases), Stage III (22 cases) and controls. Values expressed in mg per 100 ml.

IgG immunoglobulin concentrations could be related to the different stages in the progress of the cancer process. The IgM immunoglobulin levels, on the other hand, do appear to be age dependent, as their tendency is to decrease with advancing age. Our series revealed the same trend, i.e. IgM immunoglobulin levels were lowest in stage III patients, which group comprised predominantly elderly females (mean age 59 years). The average IgM level in 18 females in the control series, in which the lowest values were noted, was 88 mg/100 ml. This figure is identical with the value observed in JOHANSSON's oldest group of

patients, and it closely approximates the value for elderly females described by NORBERG.

When comparing the values for IgG, IgA and IgM immunoglobulin for the three healthy control groups (Table 1), differences were found in the immunoglobulin values of the various groups. A possible explanation of the observed differences might be the use of different standard sera used for determination of the absolute value of the reference sample. We observed that the IgG, IgA and IgM immunoglobulin values in the control group of the present investigation are close to the value for healthy adult females reported by STROOP et coll. The reported immunoglobulin levels in their series were as follows: IgG =  $1\ 064 \pm 287$  mg/100 ml; IgA =  $174 \pm 74$  mg/100 ml; IgM =  $109 \pm 41$  mg/100 ml. STROOP's method for the determination of immunoglobulin levels calls for standard sera similar to our own (STROOP et coll. 1969).

A comparison of the serum immunoglobulin levels in the three different stages in patients with cervical carcinoma with those of healthy controls reveals higher immunoglobulin IgG levels in the earlier stages of the disease. The serum level of IgG immunoglobulin is dependent upon the intensity of antigenic stimulation and on the functional capacity of the antibody producing mechanism (ANDERSEN 1966). Consequently, the increase in IgG immunoglobulins in stage I cervical carcinoma might be the result of intensive antigenic stimulation. Inasmuch as the rate of degradation of IgG immunoglobulins appears to be directly proportional to its serum concentration, a decreased rate of degradation cannot be invoked to explain the elevated IgG immunoglobulin levels. It has been observed, however, that IgG immunoglobulins are catabolized at a faster rate in cancer patients than in healthy individuals (TEE & WATKINS 1967). Decreased IgM immunoglobulin concentrations have been reported in Burkitt's lymphoma (NGU et coll. 1966), in Hodgkin's disease (GOLDMAN & HOBBS 1967) as well as in hepatomas (MCKELVEY & FAHEY 1965) and in other solid tumours (DOSTÁLOVÁ et coll. 1970). Decreased concentrations have also been observed in healthy elderly women (JOHANSSON et coll. 1968). Since the majority of stage III patients are in the older age group, the mechanism responsible for decreased concentrations of IgM immunoglobulins is more likely related to immunologic deteriorations occurring with age, than it is to the disease per se. In contrast to the observed changes in IgG and IgM immunoglobulins, IgA immunoglobulin levels remain fairly constant regardless of the stage of the disease. Increased levels of IgM immunoglobulins have been observed in long term survivors of carcinoma of the lung, for example (KRANT et coll. 1968). In the sera of patients investigated in the present report, increased levels of IgA immunoglobulins were observed, as compared with those of healthy controls; the differences, however, were not significant.

The present investigation is confined to the determination of immunoglobulin levels by means of radial immunodiffusion in patients with cervical carcinoma. From these data it cannot be concluded whether the observed differences in IgG immunoglobulins, are due to alterations of the patient's immunologic status, to antigens in some way associated with malignant disease, or to some other reason. The changes occurring in IgG and possibly IgM immunoglobulins, on the other hand, do seem to be related to the extent of the disease.

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### SUMMARY

The levels of immunoglobulins IgG, IgA and IgM were measured in seventy-seven untreated patients with carcinoma of the uterine cervix. The results were correlated with the clinical staging of the disease. Compared with control values immunoglobulins IgG were elevated in stage I of the disease ( $p < 0.01$ ). In the stage III group the mean concentration for the immunoglobulin IgM was below the value for the control series ( $p < 0.01$ ). These changes were statistically significant. In all three stages, levels for IgA immunoglobulin were slightly elevated as compared with the healthy control; these differences were, however, not significant.

### ZUSAMMENFASSUNG

Die Konzentration der IgG, IgA und IgM Immunglobuline wurde bei siebenundsiebzig unbehandelten Patienten mit Carcinoma Colli uteri gemessen. Die Ergebnisse wurden mit dem klinischen Stadium der Krankheit verglichen. Im Vergleich zu der Kontrollgruppe ist die Konzentration der IgG Immunglobuline im Stadium I der Krankheit erhöht ( $p < 0,01$ ). Im Stadium III wurde festgestellt, dass die durchschnittliche Konzentration für IgM Immunglobuline unter der Konzentration in der Kontrollserie war ( $p < 0,01$ ). Diese Unterschiede sind statistisch signifikant. In allen drei Stadien war die Konzentration der IgA Immunglobuline etwas erhöht im Vergleich zu der Kontrollgruppe, diese Unterschiede sind jedoch nicht statistisch signifikant.

### RÉSUMÉ

Les taux d'immunoglobulines IgG, IgA et IgM ont été mesurés chez soixante-dix-sept malades atteintes de cancer du col de l'utérus non traité. Les résultats ont été mis en corrélation avec le stade clinique de cette affection. Comparées aux taux des témoins, les immunoglobulines IgG sont élevées au stade I ( $p < 0,01$ ). Dans le groupe du stade III, le taux moyen de l'immunoglobuline IgM est inférieur au taux de la série témoin ( $p < 0,01$ ). Ces différences sont statistiquement significatives. Aux trois stades, les taux d'immunoglobuline IgA sont un peu élevés par rapport aux témoins sains, mais ces différences ne sont cependant pas significatives.

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