Autologous and allogeneic cell reactivity in cancer of the stomach and colorectum

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Reacción linfocitaria mixta autóloga y alogénica en pacientes con cáncer gástrico y colorrectal

Se investigó la reacción linfocitaria mixta frente a células autólogas y células alogénicas utilizando linfocitos T y no T precultivados en pacientes con cáncer de estómago o colorrecto. Los resultados demuestran que frente a antígenos autólogos ambos grupos de pacientes reaccionan con un patrón similar de respuesta, mientras que la reactividad frente a antígenos alogénicos estuvo sólo alterada en los pacientes con cáncer de colon en comparación con el grupo con cáncer gástrico. Ambas funciones fueron moduladas por el suero autólogo de cada paciente, siendo significativa la inhibición observada en la alorreactividad de los pacientes con cáncer de colon. Se discuten las características inmunomoduladoras de ambos tipos de cáncer.

Self and non-self reactivity in patients with cancer of the stomach or the colorectum were studied utilizing precultured T and non T lymphocytes. Results showed that both groups reacted to autologous antigens with a no distinctive pattern while the responses to allogeneic antigens were impaired in the colon cancer patients compared to the gastric cancer group. Either function was highly modulated by the autologous patient serum which was significantly inhibitory in the colon cancer patients alloreaction. Immunoregulatory characteristics from each cancer type are discussed.

INTRODUCTION

In vitro studies exploring the immune competence in patients bearing solid tumors have shown alterations in cellular immune responses. Adenocarcinoma of the stomach and colorectum have been included in the types of malignant tumors which might compromise the immunological reactivity[1,2]. However, only a variable percentage of patients suffering this cancer types lost lymphoproliferative capabilities during the evolution of the disease stages[3,4]. In some cases T cell functional recovery has been observed after tumor removal, phenomenon also noted in long-term survivors from advance stomach or colorectum cancer[1,5].

In the present study we have investigated the immunological recognition competence for self and non-self antigens in cases of gastric or colon cancer. Using precultured cell preparations we found that only a minority of gastric cancer patients hyporesponded to autologous or allogeneic stimuli while the colon cancer group showed a self-reactivity mostly preserved but with low alloreactive function.

These capabilities were highly modulated by the presence of each patient's serum which severely impaired the non-self reactivity from the colon cancer group with no significant influence on the self-recognition function.

MATERIAL AND METHODS

Twenty untreated well-nourished patients, mean age 55 years, 10 with stomach adenocarcinoma stage IV and 10 with colorectal adenocarcinoma stage II were carefully selected[6]. Diagnosis was made by means of clinical, radiological, endoscopical and morphological procedures. Thirty matched, healthy subjects were simultaneously explored.

T-cell preparation

Peripheral blood mononuclear cells were isolated by centrifugation on Ficoll-Hypaque gradient[7] and further fractionation into T-lymphocytes and non-T cells by rosetting with sheep red blood cells (SRBC)[8]. The T-cell fraction obtained was 90 % E-rosette positive and 95 % CD3 positive[9]. Non-rosetting cells were further purified by re-rosetting with SRBC and subsequent density gradient sedimentation until a non T cell preparation with only 5 % T cells contamination was obtained. Non T cells were treated with 25 µg/ml of mitomycin C for 30 minutes at 37 °C before using as stimulator in autologous mixed lymphocyte reaction (AMLR). Pooled non T cell fractions mitomycin treated from unrelated normal individuals were used as stimulating cells in the allogeneic mixed lymphocyte reaction (alloMLR).

Cell-preculturing

T and non T cell preparations were adjusted to 1×10^6 cells per ml in RPMI-1640 medium supplemented with 2 % pool heat-inactivated normal human serum, 25 mM hepes buffer, 2 % mM L glutamine and 1 % Penicillin-Streptomycin mixture. Before setting up the assays, both cell suspensions were incubated for a period of 18 hours, at 37 °C in an atmosphere of 5 % CO_2 air mixture[10].

Mixed lymphocyte reaction with autologous (AMLR) and allogeneic cells (AlloMLR)

Both assays were performed as described elsewhere[5,11]. The experiments were set up with 10 % final concentration

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cant difference upon comparing patient and control RPI values (table II). Moreover, a striking suppressive effect was observed when PS was added to the respective alloMLR experiment; the allo-reactivity significantly dropped not only in the patient group but also in the precultured T cells obtained from healthy subjects and which were tested simultaneously in the presence of patient serum (table II).

CIC

Five cases of gastric cancer presented CIC levels that were higher than the upper limit value (range: 50-125 µg/ml) but no correlation with the suppressing serum effect was found. Only one patient with colon cancer showed high CIC levels (325 µg of AHG/ml), mildly modified AMLR by the addition of the patient serum and normal response in AlloMLR which was suppressed by the patient serum (RPI 0.91 *vs* 0.15).

DISCUSSION

The *in vitro* mononuclear cell function explored by utilizing precultured cell preparations has provided new insights in the understanding of chronic diseases which might alter immunological lymphocyte reactivity[14, 15]. Differences in the proliferative ability between fresh and precultured lymphocytes have been observed in a wide variety of pathological states including infections and malignant entities[10, 16]. The *in vitro* immunomodulatory influences of preculturing as well as serum factors have recently been discussed[17]. The preculturing seems to avoid the presence of outer cell membrane factors which are able to block certain lymphocyte capabilities[14, 17]. Most of the accumulated data regarding immunological competence from patients with malignant disease have been obtained by means of standard mononuclear cell preparations. By utilizing precultured T and non T cell fractions we have found that the majority of the patients with cancer of the stomach or colorectum are able to mount an *in vitro* immune response to autologous antigens. The different cell subsets which participate in the immunoregulatory model represented by the AMLR have recently been dissected and discussed[18, 19]. Cells recoverable from the AMLR are able to mediate helper or suppresor functions, residing in the CD4 cell subset. The ability to proliferate in AMLR suggests that patients with carcinoma of the stomach or the colon still maintain activated those cells capable to recognize and process self-antigens.

Similar to a mostly preserved AMLR, precultured T cells from the gastric cancer group responded to alloantigens. The immunological study of gastric cancer has been less documented, but our results are similar to those presented in a previous report where by using precultured total peripheral blood lymphocytes, ten out of fifteen patients showed a normal response in one-way mixed lymphocyte culture[1]. In contrast, the cases of colon cancer did show a decrease in precultured cell proliferation to alloantigens suggesting the possible presence of a defective functional T cell. Other authors have documented failure of cell-mediated immunity in colon cancer by assessing other immunological functions[2].

Both immunoregulatory models, AMLR as well as alloMLR, were modulated by the serum derived from the gastric cancer group. On both assays a bimodal effect (enhancing or suppressing) was documented. Although no statistical diference was obtained, the inhibitory action was prevalent in the alloMLR experiments. However, the remarkable suppressive serum effect was noted in the colon cancer group. These cases maintained a self-reactivity almost unmodified by the patient serum action but the non-self reactivity was significantly inhibited. This action has been reported in cell responses to polyclonal mitogens in the presence of autologous plasma from colon cancer patients[20]. Moreover, the inhibitory serum action from the colon cancer group was also exerted in the alloreactive function of precultured lymphocytes derived from healthy subjects, suggesting a non-specific modulation of the T cell function. The possibility that high levels of CIC could partly be responsible for suppressive serum effect remains unclear; once measured by Clq solid phase only one patient from the total group of cases presented increased CIC levels and a strong blocking action on alloMLR.

A defective T cell-function to non-self antigens plus an inhibitory serum action appears to be dominant in colon cancer over gastric carcinoma, both maintaining a similar reactivity pattern to self-antigens. It seems essential to investigate other possible nature of immunomodulating serum factors which might be different depending upon the tumor lineage origin.

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