ROLE OF HOMOLOGY IN THE IC SUBREGION OF THE H2 COMPLEX IN MUTUAL SUPPRESSION OF NATURAL KILLERS AND TUMOR CELLS

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The cytotoxicity of natural killers (NK) is controlled by the D region of the H2 complex, and with rare exceptions, NK preferentially attack syngeneic and allogeneic targets rather than xenogeneic targets [4-6, 8, 14], and attack tumor cells (TC) *in vitro* in preference to those growing *in vivo* [13].

The writers have shown [3] that mouse leukemia and sarcoma cells and blood cells from patients with lymphatic leukemia are cytotoxic relative to NK targets (K-562 and EL-4 cells, maintained *in vitro*). A later investigation showed that different mouse TC are cytotoxic against lymphocytes homologous for the IC subregion of the H2 complex, in which protein synthesis was suppressed and membrane rapair disturbed [2].

Arguments in support of the possibility not just of unidirectional (NK → tumor cell) interaction between NK and TC, but also of harmful interaction in the opposite direction thus appeared.

The aim of the present investigation was to achieve conditions close to those existing *in vivo*, i.e., to investigate interaction between NK and TC transplantable into animals, in order to study two problems: 1) do NK and TC maintained *in vivo* interact with one another and how does this interaction affect the cytotoxic function of the partners; 2) what is the role of the products of the principal histocompatibility complex in this interaction?

EXPERIMENTAL METHOD

A three-component model *in vitro* was used for the investigation. It consisted of two types of effector cells and standard target cells (leukemia EL-4, $H2^b$ cells adapted for conditions *in vitro*). Mouse splenocytes were used as effector NK. Removal of macrophages from a suspension of splenocytes did not affect the level of cytotoxicity of the NK [1]. Mouse leukemia and sarcoma cells served as effector TC. The tumors and mice acting as NK donors are listed and their genetic characteristics given in Fig. 4.

As the writers showed previously [1, 3], both types of effectors separately injure the membrane of the target cells (Fig. 1, continuous arrows). The broken arrows in Fig. 1 represent interaction whose existence and nature had to be established by the present investigation. Essentially in the experiments two parameters were changed in the model system: the genotype of NK and the effector TC and the concentration of the latter (Figs. 2-4). Injury to the membrane of the target cells was tested by our modification of Namaoka's method [1].

EXPERIMENTAL RESULTS

As Fig. 2a shows, in a strictly syngeneic system ($H2^b$), in which the ratio of NK to EL-4 target cells to EL-4 effector cells was 50:1:100 (Fig. 4, line 1), the cytotoxicity index was significantly (by 59%) lower than in the complete absence of EL-4 effector TC (41%), and especially if they were present in excess (82% when the ratio was 50:1:200). If the effector

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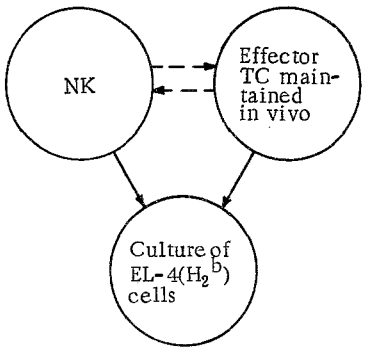

Fig. 1. Three-component experimental model (scheme).

cells were replaced by sarcoma MCh-11 cells the curve (Fig. 2b; Fig. 4*, line 2) characterizing changes in the cytotoxicity index as a function of concentration of effector TC, was similar in shape. EL-4 and MCh-11 cells are identical with respect to the H2 complex and their differences on a genetic basis are minimal.

Meanwhile, when two variants of allogeneic combinations of NK and TC were used (Fig. 2a; Fig. 4, lines 3, 4, and 5) there was no decrease in the cytotoxic index and the shape of the curves indicated summation of the cytotoxic action of the NK and effector TC on the target cells. Curves of a similar character were found (Fig. 2b) in the case of mismatching of NK and effector TC with respect to the H2 complex and complete or almost complete identity on a genetic basis (Fig. 4, lines 6 and 7).

The fall in the cytotoxicity index in a syngeneic system could have three causes: 1) specific "cold" inhibition of the cytotoxicity of NK by an excess of syngeneic TC; 2) nonspecific inhibition of the cytotoxicity of NK by an excess of unlabeled cells, creating a mechanical obstacle in the way of contact between NK and labeled targets; 3) active suppression of the cytotoxicity of NK by effector TC and vice versa (mutual suppression).

The first two causes are contradicted by the experimental results given above. In a strictly syngeneic system an increase in the concentration of unlabeled EL-4 and MCh-11 cells from 50:1:100 (corresponding to the minimal cytotoxic index) to 50:1:200 leads not to any further decrease in the cytotoxic index (expected in the case of "cold" inhibition), but to its rapid rise. The hypothesis of inhibition, whether due to the purely mechanical action of allogeneic TC or to "recognition" by natural killers of certain antigens on their surface is invalid because, with an increase in the concentration of allogeneic TC the cytotoxic index rises proportionally.

It seems unlikely that there are any other causes of the decrease of the cytotoxic index in the case of syngeneic NK and effector TC. In fact, with a completely different combination (NK from B10.D2 mice, L-1210 leukemia effector cells, and the same EL-4 target cells cultured *in vitro*) with a ratio of 50:1:100, a marked fall in the cytotoxic index also was observed (see Fig. 2b and Fig. 4, line 8). It can be concluded that syngeneic NK and effector TC, if present in a certain ratio, inhibit the cytotoxicity of each partner against the third participant — the target cells.

Coincidence or mismatching for H2 antigens affects the value of the cytotoxic index, as follows also from [2], but in a three-component system the effect of mutual suppression of NK and syngeneic TC maintained *in vivo* is manifested clearly. At the origin of each curve (with syngeneic effectors) cytotoxicity of NK against EL-4 targets is recorded in a pure form. With a ratio of 50:1:100 maximum suppression of cytotoxicity of both NK and tumor effectors was observed. At the final point of the curves, with effector TC in excess (50:1:100), cytotoxicity of the latter predominated.

In the second part of the investigation an attempt was made to answer the question: What is the role of products of the various subregions of the H2 complex in the phenomenon of mutual suppression? As will be clear from Fig. 3a and b (see Fig. 4, lines 9-13), coincidence of mutually acting cells for the K, IA, IB, IJ, and IE subregions of the H2 complex was insufficient, whereas coincidence for the subregions of the D end (Fig. 3b; Fig. 4, lines 14 and

*The cytotoxicity index shown on all lines in Fig. 4 is for a ratio of NK to target cells to effector TC of 50:1:100.

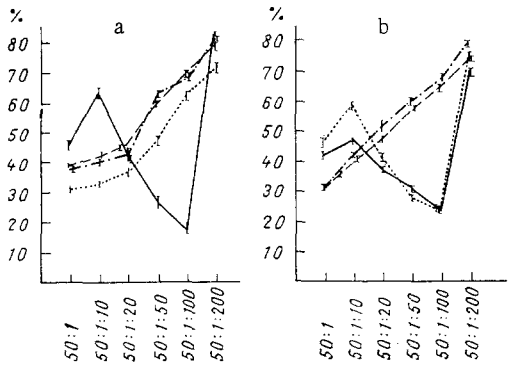

Fig. 2. Mutual suppression of cytotoxicity of NK and effector TC: a) in syngeneic system and summation of cytotoxicity in allogeneic system. Here and in Fig. 3a, b, c: abscissa, ratio NK:target cells:TC; ordinate, cytotoxicity index (in %). Continuous line) NK of C57BL/6 mice, TC from EL-4; broken line) NK from C57BL/6 mice, TC from L-1210; line of dots and dashes) NK of CBA mice, TC from EL-4; dotted line) NK from CBA mice, TC from MCh-11. b) Complete coincidence with respect to H2 complex and partial or complete mismatching on a genetic basis, summation of cytotoxicity in the case of partial or complete coincidence on a genetic basis and complete mismatching relative to the H2 complex. Continuous line) NK from C57BL/6 mice, TC from MCh-11; line of dots and dashes) NK from B10.SM mice, TC from MCh-11; dotted line) NK from D10.D2 mice, TC from L-1210.

15) was sufficient for mutual suppression of the two effectors. Evidence of suppression of the cytotoxicity of effector TC if identical with the NK for the D end (Fig. 4, line 14), and, conversely, summation of their cytotoxicity with that of NK in the case of mismatching for the D end of the H2 complex (Fig. 4, line 13) is given by the results of the next separate experiment. The cytotoxic index was compared for the following ratios between NK, EL-4 targets, and effector TC — 50:1:100, 50:1:0, and 0:1:100. In the case of coincidence for the D end the cytotoxicity index was 16 ± 0.3, 33 ± 1.3, and 44 ± 0.1% respectively, whereas in the case of mismatching it was 70 ± 1.4, 36 ± 0.3, and 44 ± 0.1%.

Coincidence for the D region alone is insufficient for suppression to take place, but in the case of mismatching for the D region alone suppression was well marked (Fig. 3; Fig. 4, lines 16-18). Finally, the phenomenon of mutual suppression was clearly manifested in the case of identity of the NK and effector TC for the IC and S subregions of the H2 complexes only (Fig. 3b; Fig. 4, line 19).

The S subregion does not code for cell surface molecules and can be disregarded. A further study of the fine genetic structure of the D end of the H2 complex may perhaps force some amendments to our conclusion regarding the unique role of the product of the IC subregion in the phenomenon of mutual suppression of cytotoxicity of normal killers and syngeneic TC. The nature of the interaction lying at the basis of the mutual syngeneic suppression described above, brought about with the aid of the product of the IC subregion of the H2 complex, must await further analysis.

The importance of this effect can be estimated in the following essential factors: 1) the presence of a receptor in NK that "recognizes" certain antigens [10]; 2) the existence of genetic control over NK activity, coded by the D-region of the H2 complex [4, 6]; 3) activation of the effector system of NK by interferon [11, 12]; and 4) the sensitivity of the targets to NK, which is associated in particular with the ability of the membrane to undergo repair [7].

The interaction described above can be tentatively regarded as cytostatic and not cytotoxic, for, judging from data in the literature [9], complete restoration of the function of one partner (NK) is possible.

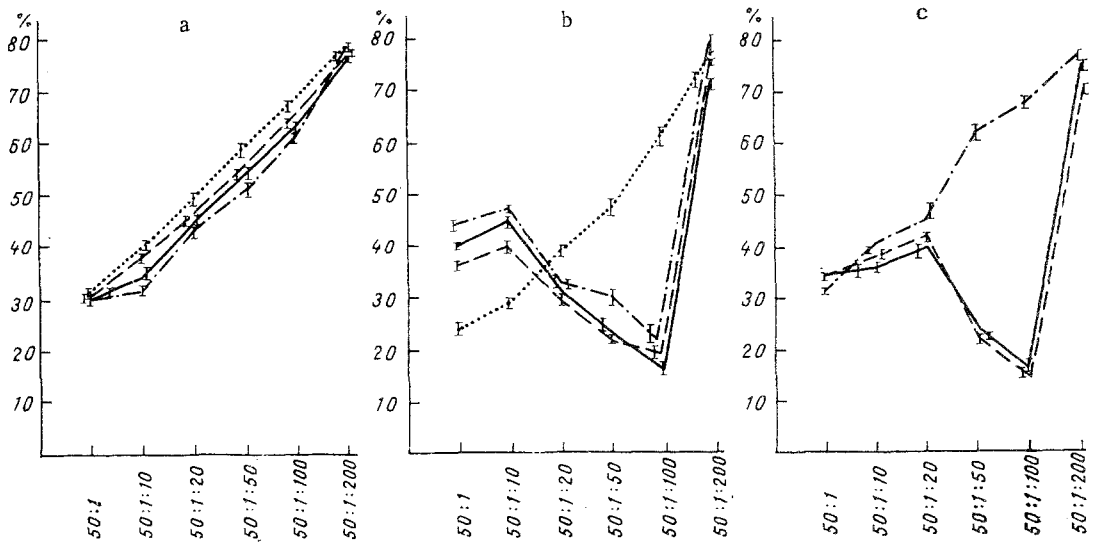

Fig. 3. Mutual suppression or summation of cytotoxicity of NK and TC depending on coincidence or mismatching for different subregions of the H2 complex: a) summation of cytotoxicity of NK and effector TC in the case of mismatching for D end of H2 complex and coincidence for K, IA, IB, and II subregions. Dotted line) NK from B10.A (5R) mice, TC from LE-4; continuous line) NK from B10.A (3R) mice, TC from MCh-11; broken line) NK from B10.A (5R) mice, TC from MCh-11; line of dots and dashes) NK from B10.A (3R) mice, TC from EL-4. b) Mutual suppression of cytotoxicity of NK and effector TC in the case of coincidence for whole D end of H2 complex and mismatching for K end, and also in the case of coincidence only for IC and S-subregions; suppression absent in the case of coincidence for K end and mismatching for D end. Line of dots and dashes) NK from B10.D2 (R101) mice, TC from SA-1; continuous line) NK from BALB/c mice, TC from SA-1; broken line) NK from A/Sn mice, TC from L-1210; dotted line) NK from CBA mice, TC from SA-1. c) Mutual suppression of cytotoxicity of NK and effector TC in the case of mismatching for D region only and summation in the case of coincidence of only this region of H2 complex. Broken line) NK from B10.D2 (R107) mice, TC from EL-4; continuous line) NK from B10.D2 (R107) mice, TC from MCh-11; line of dots and dashes) NK from B10.D2 (R107) mice, TC from L-1210.

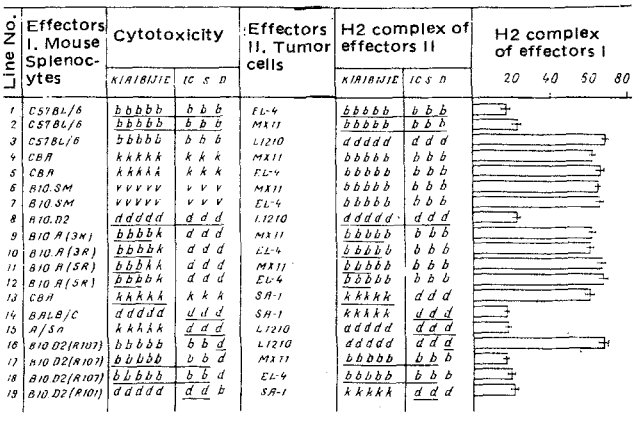

Fig. 4. Genetic analysis of mutual suppression of natural killers and tumor cells.

It can be postulated that injury to the membrane, recorded in the test with [^{3}H]uridine, can be repaired [7] and does not lead to death of the target. This effect can be conventionally called membrane toxicity.

It remains to be explained to what extent "reparable" membrane toxicity may lie at the basis of the cytostatic action of cells and, in particular, at the basis of mutual supression of NK and syngeneic TC.

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