

Cellular composition for the treatment of human or animal
organisms

The present invention relates to a cellular composition
for the treatment of human or animal organism.

5 It was very recently established, by various scientific
teams, that the local injection, in organisms affected by a
tumor, of syngenic tumoral cells secreting an interleukin
allowed the rejection of these tumors by the organism.

This was demonstrated for interkeukin-2 by Bubenik et al
10 (Immunology Letters, 19: 279-282 (1988), Immunology Letters,
23: 287-292 (1989) and confirmed in particular by Fearon et al
(Cell., 60: 397-403 (1990)) and by Ley et al (European Journal
of Immunology, (1991); 21:851-854 Res. Immunol. (1990) 141:
855-863).

15 The authors of these articles mention that rejection is
accompanied by a memorization of the response. The animal is
thus vaccinated against the subsequent growth of a tumor of
the same type, even if this has been grafted to a different
site.

20 Syngenic cancer cells producing interleukin-4 have also
been tested with identical results, as reported by Golumbek
(Science, 254: 713-716 (1991)) and Tepper et al (Cell., 57:
503-512 (1989)) as well as cells secreting the tumor necrosis
factor (TNF) as described by Blankenstein et al (J. Exp. Med.,
25 173:1047-1052 (1991)).

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The systems described in these publications nevertheless present drawbacks to their use in human therapy.

In all these publications, in fact, the cells secreting the interleukins are cells of the individual or of a syngenic individual, which have been modified to express interkeulin.

In human therapy, the major drawback of this methodology is that the cells expressing interleukin and injected into the organism are liable to continue to grow even after the tumor has been rejected.

A second drawback of the techniques described in the prior art is the method of insertion of the DNA coding for interleukins, which is often based on the use of viral and particularly retroviral vectors.

These methods have a high effectiveness of DNA transmission, but the use of virus and particularly retrovirus could present serious drawbacks in connection with human therapy.

These methods also often involve a rigorous selection of the transfectants over a long period of time, and are accordingly difficult to apply on a large scale in human therapy in particular.

To the best knowledge of the applicants, the prior art hence does not provide reliable techniques, easy to apply, and compatible with the requirements of human health, allowing to treat tumors or cancers by cells synthetizing interkeukins.



The main problem resides in a possible survival and a possible growth in the treated organism of the injected cells coding for the interleukins or of viruses derived from viruses used as vectors.

5 The applicants are therefore concerned with the use of compositions allowing to treat transiently human or animal organisms by biologically-active substances without the above-mentioned drawbacks.

10 They have demonstrated in an unexpected manner that use could be made of lines of non-syngeneic cells, and particularly of lines of allogeneic cells secreting biologically-active substances, to treat said organisms. They have thus demonstrated, in particular, that the use of non-syngenic cells allowed a transient secretion of interleukin in
15 said organisms.

 The present invention therefore has for object a composition for use in the treatment of human or animal organisms, comprising cells expressing genes enabling them to secrete in vivo one or more biologically-active substances,
20 characterized in that said cells have genetic features preventing them from growing durably in the treated organism and making them liable to be eliminated artificially or naturally from the organism.

 These biologically-active substances can in particular be
25 intended to treat transiently organisms affected by a tumor or a cancer, in which case said substances may be interleukins.

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The cells are accordingly selected to as to be eliminated after the disappearance or during the regression of the tumor or of the cancer.

These substances may also be molecules capable of
5 inducing an immune reaction of the humoral or cellular type, such as, for example, the antigen HbS described in French Patent No.80.09.041, a fragment of glycoprotein from the envelope of HIV virus or any other antigen of viral or bacterial origin, or even any normal or mutant antigen
10 involved in pathologies, for example, tumor-specific antigens, or involved in auto-immune diseases, or even antibodies or derivatives of specific-antibodies. Besides their use in the field of vaccination or immunotherapy, these cells can also help to deliver transiently other active substances, such as
15 hormones, growth factors or their fragments.

The cells are selected so that the treated organisms have an immune system allowing their elimination. Thus, the cells are not notably syngenic, but are at least partially allogeneic. The expression 'cell at least partially
20 allogeneic' means a cell which is distinguished from its host organism by at least one HLA determinant.

The cells may also be xenogeneic, although this type of cell may present the drawback of being rejected more rapidly and of secreting a smaller amount of substances.

25 But it is also possible to modify the allogeneic or xenogeneic cells to make them express antigen characteristics



of human cells, for example Class I or Class II HLA antigens, and to give them partially syngenic characteristics so as to stimulate transiently the characteristic immune responses of the host.

5 A particularly suitable cell line is the VERO line issuing from a species of monkey. In fact, these cells offer the advantage of having been designed and used by several teams (see in particular VERO cells, Origin, Properties and Biomedical Applications, Bunsiti Simuzu and Toyozo Terasima,
10 published by the Department of Microbiology of the Medical School of the University of Chiba (Japan)). Thus, their genetics are fairly well known, making it possible to reduce the risks of infection due to endogenous viruses or retroviruses. This is particularly advantageous in connection
15 with human therapy.

 The cells of the composition according to the invention may also be sensitive to a drug, thus facilitating their elimination by the introduction of said drug into the organism. One such drug may be gancyclovir, to which the cells
20 carrying the gene of thymidine kinase of the herpes virus are sensitive.

 Said immunomodulators may be in particular IL-2, IL-4, TNF (tumor necrosis factor), gamma interferon, and/or GM-CSF (granular monocytic colony stimulating factor).

25 The cells may secrete these substances alone or in combination. These substance are preferably produced in



synergistic quantities. Advantageously, such a composition secretes IL-2 and IL-4 in synergistic quantities.

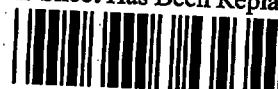
Furthermore, the cells of this composition can carry an easily-identifiable dye marker. This could be, for example, a
5 gene coding for luciferase or β -galactosidase.

To reinforce the transient character of the expression of the biologically-active substances and in particular of the immunomodulators in the treated organisms, the genes enabling the cells to secrete these substances can be introduced into
10 the cells by transfection, and particularly by transfection without subsequent selection of stable transfectant. A pool of cells is obtained, mainly composed of cells transfected by DNA, in which the latter is not integrated. In this way, the genes, and particularly those coding for the interleukins, are
15 expressed, but their corresponding DNA is rapidly eliminated during the division cycles. This helps to reinforce the transient character of the expression of the substances in the treated organisms.

Transfection consists of the introduction of nu DNA into
20 cells. This is a technique known in itself and described in particular in the technical manual of Maniatis et al (Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 1982).

One can in particular make use of precipitation with
25 calcium phosphate, electroporation, and occasionally preparations of liposomes such as the commercial preparation

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Lipofectin Reagent (Bethesda Research Laboratories, Life Technologies Inc).

The genes carrying the immunomodulators can be obtained by cloning, from cell DNA in particular, or by synthesis. For
5 IL-2 and IL-4 in particular, the DNA preparations described in Karasuyama et al (Eur. J. Immunol. 18 97 (1988)) can be employed.

Furthermore, the cells used in the composition according to the invention can possess genes enabling them to secrete
10 specific antigens of the tumor or of the cancer to be treated, in order to increase the response of the organism to these antigens.

Further quantities of tumor antigen can also be introduced by addition to the cells secreting the interleukins
15 of chemically-synthesized antigen.

The composition according to the invention may comprise several cell types, each cell type expressing an immunomodulator, or may consist of a single cell type secreting one or more immunomodulators. The invention also
20 relates to any treatment in which an active substance can be usefully delivered in vivo transiently by manipulated cell lines that are at least partially allogeneic or xenogeneic.

For reasons of simplicity of use, it may also be interesting to prepare cell lines which only express a single
25 interleukin. However, it may also be advantageous for the same cells to secrete combinations of interleukins.



Furthermore, the present invention relates in particular to the use of the cells thus defined for the fabrication of a medicinal product for the treatment of human or animal organisms affected by a tumor or a cancer, as well as to
5 medicinal products or vaccines containing said cells.

It should be noticed, that such compositions are preferably intended for use in the form of local injections, but are also susceptible to systemic use. Repeated injections can be administered, although their efficacy could deteriorate
10 as the number of injections increases, owing to accelerated rejection by the immune system.

The quantities of interkeukins produced by these cells must be adjusted according to the type of tumor. As an example, an expression of about 4000 international units of
15 IL-2/ml. 10^6 Cells is effective in the cases described below.

Nevertheless, for slow-growing tumors, smaller doses can be effective.

The present compositions are preferably used in the treatment of solid tumors, but can also be used in the
20 treatment of any other type of tumor or cancer.

The present invention is illustrated, but without limitation, by the following embodiments in which:

Figures 1A to 1E are curves illustrating the growth of tumor cells in the absence of allogeneic cells (Figure 1A), in
25 the presence of allogeneic cells not synthesizing IL-2



(Figures 1B and 1C), and in the presence of allogeneic cells secreting IL-2 (Figures 1D and 1E).

Figures 2A to 2I show the effect of cells secreting interleukins on the rejection of freshly-implanted Lewis tumors. Figure 2A is a control without injection of any allogeneic cell. Figures 2B to 2D correspond to the injection of increasing doses of P815 non-transfected cells. Figures 2E to 2G correspond to the injection of P815 cells secreting IL-2, while Figures 2H and 2I correspond to the injection of P815 cells respectively synthesizing IL-4 and a combination of cells synthesizing IL-2 and IL-4.

Figures 3A to 3C relate to the growth of Lewis tumors (y-axis) in C57Bl/6 mice as a function of days post injection (x-axis); after inoculation by Lewis tumor cells alone (Figure 3A), Lewis tumor cells in the presence of non-transfected rMTC (Figure 3B), and of rMTC transfected by IL-2 (Figures 3C).

EXAMPLE 1

Use of allogeneic cells secreting IL-2

Transformed L cells of H-2^k haplotype expressing IL-2 and called LMI (IL-2) were isolated.

The LMI cell line of mice expresses the ICAM-1 adhesion molecule and is derived from L cells described in French Patent No.80.09.041.

The LMI line was described by Christian Jaulin (Analyse structurale et fonctionnelle des antigènes

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d'histocompatibilité de classe I, Doctoral Thesis at the Université de Paris XI, 1991).

DBA/2 mice of H-2^d haplotype are injected with a mixture of $5 \cdot 10^5$ P815 cells and 10^6 or $5 \cdot 10^6$ LMI (IL-2) cells.

5 Figures 1A to 1E illustrate the results obtained.

These figures show that the allogeneic LMI cells expressing IL-2 provide higher protection against the growth of co-injected P815 cells than that provided by LMI cells not expressing IL-2.

10 The operating of Figures 1A to 1E are the following:

- . Figure 1A, $5 \cdot 10^5$ P815 cells,
- . Figure 1B, $5 \cdot 10^5$ P815 cells + 10^6 L cells,
- . Figure 1C, $5 \cdot 10^5$ P815 cells + $5 \cdot 10^6$ L cells,
- . Figure 1D, $5 \cdot 10^5$ P815 cells + 10^6 LMI (IL-2) cells,
- 15 . Figure 1E, $5 \cdot 10^5$ P815 cells + $5 \cdot 10^6$ LMI(IL-2) cells.

Of the five mice corresponding to Figure 1D, two are totally protected, one developed a tumor very late, and the remaining two developed tumors rapidly.

20 In the operating conditions of Figure 1E, a single mouse formed a tumor rapidly, whereas the other four were totally protected.

In Figures 1A to 1E, the figures indicated on the x-axis correspond to the number of days post injection, whereas the
25 y-axis indicates the volume of the tumor in cm³.

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EXAMPLE 2

Effect of allogeneic cells secreting interleukins against Lewis tumors

5 $5 \cdot 10^5$ isolated cells of freshly-implanted Lewis tumors (H-2^b haplotype) mixed with different quantities of P815 (IL-2) or P815 (IL-4) are injected into C57B1/6 mice (H-2^b haplotype).

The P815 cells are H-2^d haplotype.

10 Figures 2A to 2I were obtained in the following conditions:

- Figure 2A, $5 \cdot 10^5$ Lewis cells,
- Figure 2B, $5 \cdot 10^5$ Lewis cells + $5 \cdot 10^5$ P815 cells,
- Figure 2C, $5 \cdot 10^5$ Lewis cells + 10^6 P815 cells,
- 15 • Figure 2D, $5 \cdot 10^5$ Lewis cells + $2 \cdot 10^6$ P815 cells,
- Figure 2E, $5 \cdot 10^5$ Lewis cells + $5 \cdot 10^5$ P815 (IL-2) cells,
- Figure 2F, $5 \cdot 10^5$ Lewis cells + 10^6 P815 (IL-2) cells,
- Figure 2G, $5 \cdot 10^5$ Lewis cells + $2 \cdot 10^6$ P815 (IL-2)
- 20 cells,
- Figure 2H, $5 \cdot 10^5$ Lewis cells + 10^6 P815 (IL-4) cells,
- Figure 2I, $5 \cdot 10^5$ Lewis cells + $5 \cdot 10^5$ P815 (IL-2) cells + $5 \cdot 10^5$ P815 (IL-4) cells.



In these figures, the x-axis indicates the number of days post treatment, and the y-axis indicates the volume of the tumors expressed in mm³.

These results show that the P815 (IL-2) cells provide a high and reproducible protection, whereas the non-transfected P815 cells do not provide this type of protection.

The P815 (IL-4) cells (Figure 2H) also provide protection, but less than that provided by the P815 (IL-2) cells.

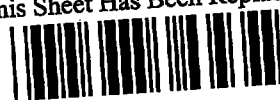
However, no synergy was observed between the effect of the P815 (IL-2) cells and the effect of the P815 (IL-4) cells (Figure 2I), but, on the contrary a rather antagonistic effect.

EXAMPLE 3

Rejection of Lewis tumors in C57B1/6 mice induced by tumor cells secreting interleukin-2.

Medullary thyroid carcinoma of the rat (rMTC) is spontaneous neoplasm derived from intra-thyroid C cells secreting calcitonin. The specific cell line obtained from these cells (rMTC 6.23) has been described (Zeytinoglu et al, Endocrinology, 107:509 (1980)).

The capacity of this strain secreting large quantities of interleukin-2 (5000 U/ml $\cdot 10^6$ cells per 24 h) to induce antitumor immune protection in a xenogeneic host has been tested. C57B1/6 mice were inoculated with either $2.5 \cdot 10^5$ Lewis tumor cells alone, or with these cells in combination with 10^6 rMTC (IL-2) rat cells.



The xenogeneic cells secreting interleukin-2 induce significant protection, as shown by Figure 3.

Figure 3A relates to the subcutaneous cells with inoculation of six C57B1/6 mice with $2.5 \cdot 10^5$ Lewis tumors
5 cells alone.

Figure 3B and 3C correspond respectively to the combination of $2.5 \cdot 10^5$ Lewis cells with 10^6 non-transfected rMTC cells (Figure 3B), or with 10^6 rMTC cells transfected by interleukin-2 (Figure 3C).

10 All the animals without tumors on the sixth day post injection did not develop any tumor after 60 days.

However, despite the demonstration of significant protection, it is nevertheless necessary in the same conditions to add four times more xenogeneic cells secreting
15 interleukin-2 than allogeneic cells.

EXAMPLE 4

Influence of the presence of NK-1.1 cells on the rejection of tumors

The effect of the selective elimination in vivo of Natural
20 Killer (NK) cells on the rejection of tumors in C57B1/6 mice co-innolated with Lewis tumor cells and allogeneic P815 (IL-2) cells was tested.

The elimination in vivo by the antibodies was carried out by intra-peritoneal injection of 100µg of purified NK-specific
25 monoclonal antibody, during three days, starting one day after the injection of the tumor cells.

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The effectiveness of the elimination of the NK cells was assessed by a chromium 51 salting-out test using YAC1 target cells (Kiessling et al, Eur.J.Immunol.,5:112(1975)), as described by Koo et al (J.Immunol., 137:3742 (1986)).

5 It was confirmed that the treatment was effective on the Natural Killer activities endogenous and induced by Poly-IC of the YAC1 cell line in the spleen of the treated animals.

The results are summarized in Table 1 below.

By comparison with untreated animals, the tumors grow more
10 rapidly in mice into which anti-NK antibodies have been injected. Moreover, the evaluation of tumor protection shows that the mice from which the NK-1.1 cells were eliminated by an antibody treatment were incapable of inhibiting the tumor growth of Lewis cells (Table 1).

15 It must nevertheless be observed that the inoculation of allogeneic cells secreting lymphokines into these treated animals produces a delay in the appearance of the tumors, and a decrease in their average volume.

EXAMPLE 5

20 Study of the rejection of Lewis tumor cells by mice who have previously rejected tumor cells of the same type

Three groups of C57B1/6 mice, on which experiments were already made on the rejection of Lewis tumor cells co-innolated with P815 (IL-2) cells, were tested six weeks
25 after the first rejection with Lewis tumor cells ($5 \cdot 10^5$).

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None of the mice tested survived these injections. However it was observed that tumor growth was slightly delayed in comparison with the growth in the control animals who had not been previously treated with Lewis tumor cells and P815 (IL-2) cells.

In conclusion, these overall results have served to demonstrate:

- that therapy by cells expressing interleukin genes is feasible for spontaneous tumors, and not only for chemically-induced tumors as described in the prior art,
- the use of allogeneic cells is feasible, and allows the rejection of the tumors,
- the delivery by transient expression of a biologically-active substance is feasible,
- allogeneic cells or partially-allogeneic cells can be used as carriers of a foreign antigen to the host capable of inducing an immune reaction of the humoral or cellular type,
- the « Natural Killer » (NK) cells are involved in the protection induced by IL-2 expressed by the allogeneic cells.

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Table 1

Growth of Lewis tumors in C57B1/9 mice
treated with an anti-NK1.1* monoclonal antibody

TREATMENT IN VIVO	CELLS INJECTED		AVERAGE VOLUME OF TUMOR (cm ³)		FRACTION OF ANIMALS PROTECTED ⁺⁺⁺
	PARENTAL TUMOR	ALLOGENEIC CELLS (5.10 ⁵)	D ₁ ⁺	D ₂ ⁺⁺	
-	Lewis	-	0.11 ± 0.07	1.76 ± 0.42	0/5
Anti-NK 1.1	Lewis	-	0.25 ± 0.18	2.2 ± 0.60	0/5
Anti-NK 1.1	Lewis	P815 (5.10 ⁵)	0.42 ± 0.20	2.30 ± 0.58	0/5
Anti-NK 1.1	Lewis	P815-(IL2)(5.10 ⁵)	0.12 ± 0.15	2.0 ± 0.7	0/5
Anti-NK 1.1	Lewis	P815-(IL4)(5.10 ⁵)	0.14 ± 0.22	2.8 ± 1.7	0/5
Anti-NK 1.1	Lewis	P815-(IL2)(5.10 ⁵)	0	1.3 ± 0.4	0/5
Anti-NK 1.1	Lewis	P815-(IL4)(5.10 ⁵)			0/5

* The percentage of specific salting-out of ⁵¹Cr chromium after incubation of the target cells for 4 h at 37°C is negligible for the treated animals, whereas, in the spleen cells induced by Poly-IC, it is 54 and 23 % after 1 day and 8 days respectively after treatment in vitro with a target cell: effector cell ratio of 1/100.

** The treatment was carried out using the antibody described by Koo and Peppard (Hybridoma, 3:301 (1984)).

+ Measurement of tumor volume 9 days after inoculation of C57B1/6 mice.

++ Measurement of tumoral volume 16 days after inoculation of C57B1/6 mice.

+++ The fraction of the animals protected measured is the fraction of animals without detectable tumors 30 days after inoculation.



CLAIMS

1. Composition for use in the treatment of human or animal organisms comprising cells expressing genes enabling them to secrete in vivo one or more biologically active substances,
5 characterized in that said cells are at least partially allogeneic or xenogeneic for the treated organisms.

2. Composition according to Claim 1, for use in the treatment of organisms affected by a cancer or a tumor, characterized in that said biologically-active substances are
10 immunomodulators.

3. Composition according to Claim 2, characterized in that the cells exhibit genetic characteristics making them susceptible to elimination after the disappearance or regression of the tumor or of the cancer.

15 4. Composition according to one of Claims 2 and 3, characterised in that said immunomodulators are IL-2, IL-4, TNF, gamma interferon, and/or GM-CSF.

5. Composition according to one of Claims 1 to 4, characterised in that the cells are sensitive to a drug
20 promoting their elimination from the organism.

6. Composition according to Claim 5, characterized in that said substance is gancyclovir, in which case the cells carry the gene of thymidine-kinase of the herpes virus.

7. Composition according to any one of Claims 2 to 6,
25 characterized in that said cells secrete the immunomodulators in synergistic quantities.

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8. Composition according to Claim 7, characterized in that the cells secrete IL-2 and IL-4 in synergistic quantities.

9. Composition according to any one of Claims 1 to 8, characterized in that the cells carry a dye marker.

5 10. Composition according to any one of Claims 1 to 9, characterized in that the genes have been introduced in the cells by transfection.

11. Composition according to any one of Claims 2 to 10, characterized in that the cells also possess genes enabling
10 them to secrete specific antigens of the tumor or of the cancer to be treated.

12. Composition according to any one of Claims 2 to 11, characterized in that the cells comprise several cell types, each secreting one or more immunomodulators and/or an antigen
15 specific to the tumor to be treated.

13. Composition according to Claim 1, characterized in that said substances are antigens capable of inducing an immune reaction of the humoral or cellular type.

14. Composition according to Claim 1, characterized in
20 that said substances are specific antigens of tumors or involved in auto-immune diseases, antibodies or derivatives of antibodies.

15. Use of the cells defined in one of the Claims 2 to 14 for the production of a medicinal product for the treatment of
25 human or animal organisms affected by a tumor or a cancer.

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16. Medicinal product or vaccine containing cells defined
in one of the Claims 1 to 14.



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