Presence of endothelial granules in the aorta of vertebrates

Class	No. of species studied	No. of species in which the granules are absent and percentage	Estimation of granules frequency in cytoplasm	Authors
Birds	4	3 (75%)	low	SANTOLAYA and BERTINI[9]
Mammals	10	1 (10%)	low	WEIBEL and PALADE[6]; LEMEUNIER, BURRI and WEIBEL[10] SANTOLAYA and BERTINI[9]
Reptiles, Amphibians, Fishes	11	0 (0%)	high	SANTOLAYA and BERTINI[9], STEINSIEPE and WEIBEL[8]

The data were prepared by pooling the results of present and previous observations made by us and other authors

The bats (*Myotis chiloensis*) were captured in the proximity of the city.

The species studied present, inspite of their peculiar evolutionary characteristics, a picture similar to that found by us or other authors in most species of the same class, as regards the presence and frequency of endothelial granules. In fact these bodies are absent in penguin, and they are found in a low number in bat and seal. The endothelial granules are absent as well in birds other than penguin, such as chick (*Gallus domesticus*) and pigeon (*Columba livia*). These 3 birds differ widely with respect to environment, flight and feeding habits; one of them underwent cross breeding and selection by man. Nevertheless, these species have in common the lack of such granules. The results of our former and present observations on endothelial granules (abdominal aorta) together with those of other authors, are shown in the Table. It appears that none of the 11 cold-blooded species gave a picture similar to that found in birds or mammals. Only one of the 10 mammals studied did not present endothelial granules. These structures were absent in 3 of the 4 birds studied.

Certainly more species must be investigated to reach a reliable conclusion; however it seems at present that endothelial granules tend to disappear in birds, their frequency is much lower in mammals than in reptiles, amphibians and fish. It is therefore tempting to consider the granules as a fine-structural aspect of evolution in vertebrates. Anyway, the data of the Table strongly suggest that important changes happened in endothelial cells through the evolutionary steps that produced warm-blooded animals[10,11].

Resumen. Se describe la ultraestructura de células endoteliales de aorta abdominal de murciélago, foca y pinguino y se discuten los resultados en función de estudios precedentes nuestros y de otros autores sobre esas células en diferentes especies. La presencia, o ausencia, y frequencia de los gránulos citoplasmáticos en las células endoteliales en las especies estudiadas parecería la expressión de un proceso evolutivo a nivel de clases.

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Release of a Cytotoxic Factor by Murine Spleen Cells in the Presence of a Tumour Allograft

The immune response is a complex phenomenon involving cellular differentiation which results in a serological immunity and in a cell-mediated immunity which play a part in numerous defence mechanisms of the organism and, in particular, in graft rejection.

During the past few years many reports have indicated the importance of soluble mediators, especially lymphotoxins, in cell-based immunity. These lymphotoxins are liberated by the secondary stimulation in vitro of lymphoid cells sensitized against molecular antigens such as tuberculin or B.G.G.[1-5] or against cellular antigens[6].

Using double compartmented diffusion chambers we have demonstrated that the spleen cells of mice which have rejected a tumour-allograft release a large amount of a soluble factor when re-exposed to the antigen. This soluble factor is cytotoxic[7] and non-specific.

On the other hand, it is known that a tumour allograft may be accepted either temporarily or permanently. In this situation it is relevant to ask whether production of the cytotoxic factor is continued, reduced or completely suppressed. In order to study this problem we have used a specific tumour from one strain of mice which, when grafted into a different strain, takes successfully in a certain number of cases.

Material and Method. The tumour concerned is a rhabdomyosarcoma induced in C3H mice by methylcholanthrene and used between the 63rd and 72nd generation

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Release of a cytotoxic factor by spleen cells in relation to the rejection or the acceptance of a tumour allograft

	Spleen cells of animals sensitized to a tumour allograft Compartment A[a]						
		Allograft rejected			Allograft accepted		
		Without antigen	With antigen	Cytotoxic index[c] (%)	Without antigen	With antigen	Cytotoxic index (%)
Experiment 1		4.8 ± 0.87[b]	0.33 ± 0.05	92.2	4.26 ± 0.98	1.89 ± 0.75	55.63
Experiment 2		4.26 ± 0.88	1.14 ± 0.31	73.23	4.92 ± 0.06	4.71 ± 1.13	4.26
Experiment 3	Target cells	2.42 ± 0.33	0.25 ± 0.05	89.6	2.93 ± 0.36	1.57 ± 0.59	29.5
Experiment 4		2.62 ± 0.96	0.55 ± 0.08	79.0	4.22 ± 0.68	2.9 ± 0.73	31.2
Experiment 5	Compartment B	3.68 ± 0.31	0.74 ± 0.29	79.8	2.26 ± 0.36	1.32 ± 0.56	41.5
Experiment 6		1.61 ± 0.41	0.30 ± 0.03	81.36	1.93 ± 0.26	1.59 ± 0.37	17.61

[a] The experiments were carried out in diffusion chambers with 2 compartments A and B. [b] Each figure represents the average of the number of target cells × 10^6/ml found in 5 diffusion chambers ± standard error. [c] Cytotoxic index represents the % of target cells killed when the spleen cells are brought together again with the antigen.

of grafting. Mice of the Swiss/B strain received a fragment measuring 1 mm^3 of this tumour which took successfully in about a quarter of the animals.

For each experiment we have compared the amount of cytotoxic factor released by the spleen cells of mice which received grafts on the same day and which either rejected or accepted the allograft. In the latter case the tumour carried by the mouse had a diameter between 2 and 4 cm.

The experiment was carried out in double compartmented diffusion chambers, the compartments A and B being identical, based on the chambers described by ALGIRE[8], and formed by joining together 2 simple diffusion chambers, the central membrane being common to both. The membranes were Millipore filters of 0.1 μm porosity and impermeable to cellular penetration.

Into compartment A was introduced 0.2 ml of a suspension of spleen cells from mice which had either accepted or rejected the tumour, in a concentration of 100×10^6 nucleated cells/ml. In some cases these cells had been re-exposed to the antigen in the form of viable tumour cells in a concentration of 2×10^6 cells/ml, and in other cases not. Target cells, taken from a chemically-induced tumour in mice of the Swiss/B strain, were introduced into compartment B in a concentration of 2×10^6 cells/ml.

When filled and sealed, the chambers were implanted under the skin of the back of the mouse. 5 days later they were removed and the target cells counted after the occasional aggregate had been broken up with the aid of a syringe. For each measurement the mean was taken of the number of target cells in 5 diffusion chambers ± the standard error.

The cytotoxic index represents the percentage of target cells killed when the spleen cells had been re-exposed to the antigen.

Results and discussion. The results are shown in the Table. It can be seen that in those mice which had rejected the tumour allograft there was a high amount of cytotoxic factor released by the spleen cells which had been re-exposed to the antigen. In the mice which had accepted the allograft, the spleen cells re-exposed to the antigen also released a cytotoxic factor but the level was much lower. The results show that there is a relationship between the amount of cytotoxic factor released and the acceptance or rejection by the mouse of the allogeneic tumour graft.

Several hypotheses may be advanced to explain these findings. The maintenance of the allogeneic tumour graft corresponds to a depression of the immunological defence mechanisms. When re-exposed to the antigen, the spleen cells of the animal partially deprived of immunity release less cytotoxic factor than those of the animal which rejected the tumour. This immunological depression could be explained by the disappearance of certain clones of immunologically competent cells capable of reacting to the antigens carried by the allogeneic tumour cells.

A further explanation might be that the animal has in its serum either facilitating antibodies or blocking factors, such as HELLSTRÖM[9,10] has demonstrated under certain conditions. These factors, by becoming attached to the lymphoid cells, might diminish their ability to react to the antigen.

If the existence of a partial depression of immunity in an animal bearing the tumour is accepted, it is pertinent to ask whether this depression is specific for the antigens carried by the tumour allograft or whether it is operative in the response to all antigens. Further experiments would be necessary to answer this question.

It must also be pointed out that our results would explain the phenomenon of concomitant immunity observed by GERSHON[11] who showed in vivo that an animal with an allogeneic lymphoma may reject a second graft of the same lymphoma. The presence of a cytotoxic factor in vivo would explain the rejection of the second tumour graft.

Résumé. Des cellules spléniques de souris, ayant accepté une allogreffe tumorale, libèrent, lorsqu'elles sont remises en présence des cellules tumorales dans des chambres à diffusion, un facteur cytotoxique soluble mais à un taux bien moindre que lorsque la souris a rejetté l'allogreffe tumorale.

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