The Protection of Mice Against Experimental Infection by Means of Immunization with Enriched Membrane Fraction of *Salmonella typhimurium*

There is a substantial difference of opinion in the literature concerning the protection that can be obtained from vaccines constituted by non-living germs or from fractions extracted from them, agreement being achieved only regarding vaccines constituted of living germs with reduced virulence.

In a previous paper, we reported data on the production of antibodies which inhibit in vitro the growth of *Micrococcus lysodeikticus* in rabbits immunized with purified bacterial membranes[1].

In the present study, the immunogenicity of living pathogenic germs and their enriched membranes fractions has been comparatively investigated in order to estimate their respective protecting effects against infection.

Salmonella typhimurium 74 NCTC grown in nutrient broth Difco at 37 °C was used. This strain gave an LD_{50} of 5×10^4 when injected s.c. in mice. Membranes were prepared by the SCHNAITMAN method[2] from cultures in the log growth phase washed 3 times in saline. Succinic dehydrogenase and lactic dehydrogenase determination was used as indication of membrane isolation[3]. The membranes fraction isolated by this method were contaminated with cell-wall lipopolysaccharides.

Salmonella-free CD-1 mice weighing about 15 g were used. The tests were repeated 10 times on 4 groups of 20 mice. Animals of the first group received every week 0.25, 0.50 and 1.0 mg of lyophilized membranes. The first inoculation was given s.c. in complete Freund adjuvant (CFA). The other inoculations were made i.m. in saline. The second group was immunized with cell lipopolysaccharides prepared as described by WESTPHAL et al.[4], according to the same schedule as the first group. The third group of animals was immunized with living cells. Two aliquots of 5×10^3 cells in 0.5 saline were inoculated at 15 day intervals. Mortality was approximately 25% so that immunized mice were survivors of a minimally virulent infection. Animals inoculated with saline alone in CFA and kept under the same conditions were used as control. A week after the last inoculation, the mice were tested for the presence of antimembrane fraction and anti-0 antibodies.

Animals of the 3 groups tested presented antibodies reacting both with cells in 0 phase and with membrane fraction. Titers were 1:320 for both antibody types, in the animals of the first group; 1:80 for antimembrane fraction and 1:1280 for anti-0 in the animals of the second group; 1:1280 or higher for both anti-membrane and anti-0 in the animals of the third group.

The immunity of mice was challanged in vivo by s.c. inoculation of 100 LD_{50} of *Salmonella*; this dose killed all the control mice within 10 days. Mortality was followed for 30 days and protection was measured as percentage of survival.

Survival was 70% ± 6 for mice treated with membrane suspension; 74% ± 6 for animals treated with live organisms and 40% ± 5 for animals treated with LPS. Statistical analysis of the results obtained from the 10 tests performed for each of the 4 groups of animals showed no significant difference between the treatments (P value based on χ^2 test < 0.05).

Results show that protective immunity of approximately the same order is stimulated by living microorganism or by membrane fraction obtained from them.

Riassunto. Topi immunizzati con membrane isolate da *Salmonella typhimurium* presentano una resistenza alla infezione sperimentale uguale a quella dimostrata da topi immunizzati con *Salmonelle* viventi.

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Local Monoclonal Immunoglobulin Production in Cancer Patient

Presence of M-component in serum of cancer patients has been described with prevalence in those bearing gastrointestinal (GI), bladder and lung cancers[1,2]. There is no distinct link between the presence of M-component and the type of cancer. The occurance of the former is apparently similar to that observed in the normal population[2].

While searching immunofluorescent stained sections of regional lymph nodes of 14 colon cancer patients, we have observed the presence of nearly 3 times as many immunoglobulin bearing plasma cells in nodes from cancer patients as compared to control nodes obtained from the vicinity of gastric or duodenal ulcers. Among the immuoglobulins produced, IgM predominated[3].

In one of the cases examined, large clumps of plasma cells showed a pattern of monoclonal immunoglobulin G (IgG) production. The afore mentioned case was a 68-year-old male, with rectal adenocarcinoma whose tissues became available after surgery. Tumor adjacent mucosa had no signs of inflammation. Plasma cells were identified in the tumor itself, surrounding mucosa and regional lymph nodes by means of a battery of monospecific immunofluorescent reagents. The relative quantity of plasma cells was determined by means of cellular density index (CDI), i.e., the mean number of cells in the field of vision under high power of microscope – ×480.

The CDI for the regional lymph nodes was very high (8.92) for IgG producing cells which constituted 90% of the total plasma cell number. The remaining 10% consisted of IgA, IgM and IgE producing cells (Table). In the case

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Examination of the number of plasma cells in lymph node and immunoglobulin levels in patient's serum

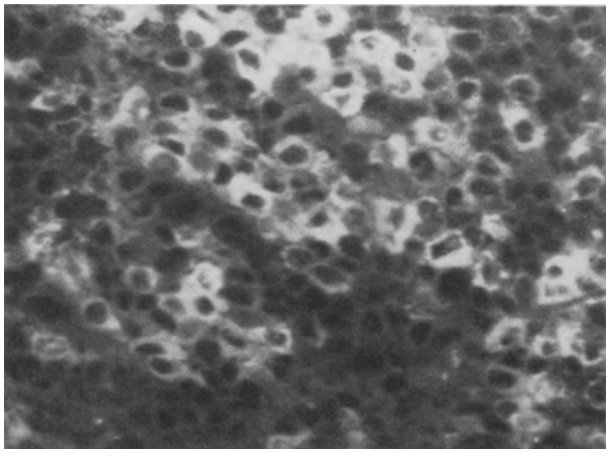
Ig	CDI[a] in lymph-node		Serum immunoglobulin levels in International Units	
	Examined case	13 cases of other colon cancer (mean values)	Examined case	Blood donors values
IgG	8.92	0.43	88	149 ± 43
IgA	0.47	0.45	68	139 ± 51
IgM	0.10	0.90	206	152 ± 48
IgE	0.33	0.06	n.t.	n.t.
K:L ratio	31:1	2.5:1	n.t.	n.t.

CDI, cell density index; n.t., not tested.

described the kappa: lambda-light chain ratio was 31:1, while in other cancer lymph nodes it did not exceed 2.5:1. Localization of kappa specific cells (Figure) corresponded closely to those of IgG and strongly favored the view that these IgG producing cells arose from single clone.

The lymph node contained a small metastatic foci, which did not change its structure. Germinal centres of lymphatic nodules were very few and inconspicious.

The patient displayed hypoproteinemia (total protein 3,4 g/100 ml). Quantitative serum immunoglobulin determinations revealed a decrease of IgG and IgA and an increase of IgM (Table). Both, paper electrophoresis and immunoelectrophoresis did not show monoclonal protein in serum.



Regional lymph node of cancer of the rectum. Specific staining of plasma cells with anti-human kappa antiserum labelled FITC. ×960.

These data show that regional lymph nodes in cancer may be a site of monoclonal Ig synthesis and that this phenomenon may not be detectable in the patient's serum.

The observed hypoproteinemia could be due to the loss of protein via the GI tract, a possibility suggested for cancer of the GI tract by JONES[4]. The failure to observe a monoclonal spike in the serum cannot be explained at this time but maybe due to the amount of IgG produced and the fact that it is produced focally.

The cause of local monoclonal immunoglobulin production and whether this Ig possesses anti-tumor antibody specificity remains unknown. Some observations hint however, that there exists a link between monoclonal immunoglobulin synthesis in the lymph node and an existing tumor. The absence of distinct inflammation in the immediate vicinity of the primary cancer site and the existence of metastatic foci as a source of antigenic material in lymph node, suggest this possibility. The described case indicates that monoclonal immunoglobulin production not related to general plasma cell dyscrasia may be manifested only on the local level.

Résumé. La technique directe de l'immunofluorescence a permis de mettre en évidence dans un ganglion lymphatique adjacent à la tumeur cancéreuse du rectum la présence de nombreuses cellules sécrétant de IgG monoclonal.

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Localizing Properties of Anti-Nervous Tissue Antibodies in Rat Cervical Ganglion

Since the demonstration that a rabbit anti-cat caudate nucleus serum affects the bioelectrical activity of caudate nucleus of the cat brain[1], the anti-nervous tissue antibody has become a valuable tool in structural and functional studies of the brain and the neuron[2]. The immunological investigations of the neuron concern, inter alia, the antigenic definition of neuronal components, and the mapping of antigens in the neuron and its microenvironment. The present report deals with the latter subject

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