Suppression of the Primary Immune Response by Antilymphocyte Serum

In the course of our systemic investigation-series concerning the mode of action of antilymphocyte serum (ALS) upon the different phases of immunological events of different types of active immunity, we recently described the immunosuppressive effect of ALS on the development of anti-helminth immunity[1]. Present paper summarizes results of our investigations concerning the suppression of the primary immune response in cases of antibacterial, respectively antitoxic immunities.

The main purpose of the present work was to determine the effect of ALS upon priming, the inductive and productive phases of developing primary immunity. As models single-shot mouse-immunization-tests were chosen.

Groups of adult female Swiss albino mice were immunized by graded doses of (1) aluminium hydroxide adsorbed *E. insidiosa* vaccine, respectively (2) aluminium phosphate adjuvanted tetanus toxoid. A 'two pulse' anti-mouse ALS was prepared using our method, i.e. rabbits were immunized twice by aluminium phosphate adjuvanted thymocytes, as described in reference[2]. The ALS did not show any signs of in vivo toxicity and exhibited a graft-protecting effect for 26 days on the average in Swiss female mice grafted by CBA mouse-tail skin grafts. The graft-protective assay was done according to the standard method of MEDAWAR's school[3].

Normal rabbit serum (NRS) was administered as control of supposed antigenic competition to ALS. Mice were injected s.c. with ALS, respectively NRS in 2 separate doses corresponding to 0.4 ml each. The timing of immunizations, ALS, respectively NRS treatment, together with the results of experiment are shown in the Table.

Challenges of the immunity grades were made by the specific noxogenic agents as follows: (1) Mice immunized with *E. insidiosa* vaccine were infected with living *E. insidiosa* germs (10^5 MLD per mouse) 2 weeks after immunization. (2) The tetanus immunity was challenged by 20 LD_{50} of tetanus toxin per mouse at the 21st day after immunization.

The degrees of actual protections against the challenging bacterium strain, respectively tetanus toxin are expressed in relative terms, i.e. in values of relative potency (RP) calculated on the basis of the ED_{50} values corresponding to the results obtained in the respective groups. On the basis of results achieved, the following effects can be recorded: The development of both types of immunity was significantly suppressed by ALS treatment performed either 2 and 5 days before or after immunization. ALS treatment prior to immunization was found more effective than the one performed already in the inductive phase of immunity ($P\% < 0.1$ resp. ~ 0.2). The phenomenon may be explained by an effective 'blindfolding' of the immunologically important cells prior to priming. In the case of ALS treatment following the immunization, a part of the adsorbed antigen already reached the immunocompetent cells and so a higher degree of immunity could develop than in the former case.

ALS treatment performed shortly before the challenges failed to alter the grades of immunities as compared with the untreated controls' effectivenesses. This phenomenon points to the fact that ALS cannot change the titer of circulating antibodies if administered in the productive phase of immunity.

A definite antigenic competition was observed between the NRS and the *E. insidiosa* vaccine. This finding proves that the protein-components of ALS may act as antigens in the course of ALS treatment. In spite of the fact that ALS – as antigen itself – may cause competition of antigens, its immunosuppressive effect cannot be reduced to this phenomenon only. The immunosuppressive effect of ALS was at least 15 times higher than the grade of inhibition caused by the competing effect of NRS. This phenomenon needs further investigation.

Code	Treatments	On days	Effectiveness of EIV[a] RP	95% confidential limits of RP	Effectiveness of TT[b] RP	95% confidential limits of RP
1	no	–	1	–	1	–
2	ALS	−5, −2	0.03	0.028–0.034	0.37	0.21–0.64
3	NRS	−5, −2	0.56	0.500–0.620	0.85	0.62–1.15
4	ALS	+2, +5	0.51	0.430–0.550	0.42	0.30–0.59
5	NRS	+2, +5	1.28	1.170–1.400	0.98	0.68–0.99
6	ALS	+10, +12	1	–	–	–
7	ALS	+16, +19	–	–	1	–

[a] EIV, *E. insidiosa* vaccine; [b] TT, tetanus toxoid; RP, relative potency; −2, treatment prior to immunization; +5, treatment following the immunization.

Zusammenfassung. Die immunosuppressive Wirkung des Antilymphozytenserums wird beschrieben.

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The Absence of Normal Serum Proteins in Lymphoma Patients Revealed by Disc Electrophoresis

By disc electrophoresis at least 25 protein bands can normally be identified in human serum on polyacrylamide gels stained with aniline blue black. Other reagents are available to aid in the detection of suggestive bands. Coomassie Brilliant Blue R-250 is such a reagent and is therefore suitable for ascertaining the absence of normal serum proteins in patients having such diseases as multiple myeloma.

Materials and Methods. 97 serum samples were collected from patients having various neoplasms. Included were 28 lymphosarcomas (LSA); 11 chronic lymphocytic leukemias (CLL); 12 chronic granulocytic leukemias

(CGL); 18 undifferentiated lymphomas (L), 5 multiple myelomas (MM); 18 Hodgkin's disease (HD), 3 acute myeloblastic leukemias (AML); and 2 giant follicular lymphomas (GFL). Controls were 33 specimens obtained from blood donors. Sera were stored at -20 °C during the collection period (about 18 months), and clarified immediately before use by centrifugation at $20{,}000 \times g$ for 20 min.

Disc electrophoresis was performed by the modified method of ORNSTEIN and DAVIS[1]. The anionic gel system provided a final concentration of 7.5% acrylamide with a running pH of 9.3.

Three reagents were used to stain protein bands: 0.02% aniline blue black in 3% acetic acid, 0.004% nigrosin in 3% acetic acid, and 0.02% Coomassie brilliant blue R-250 in 7% acetic acid. Gels were immersed overnight in each reagent and destained on each of 3 days with 7% acetic acid.

Results. The accompanying Table carries the results of this study. 1 of 2 separate bands, present in all control sera, was absent in 60 of the 97 sera obtained from patients. The 2 bands were never missing in any one patient. Band X was missing in 18% of the lymphosarcoma sera examined. Band Y was missing in 43% lymphosarcoma, 56% undifferentiated lymphomas, and 72% Hodgkin's disease.

CLARK[2] has proposed an identification system for serum protein bands based on patterns obtained with combined paper and disc electrophoresis. The patterns suggest that X and Y are α-2-globulins. This designation agrees with patterns obtained from alcohol fractionated human serum proteins in our laboratory. The mobility of band X (and band Y) is visually present in the accompanying Figure.

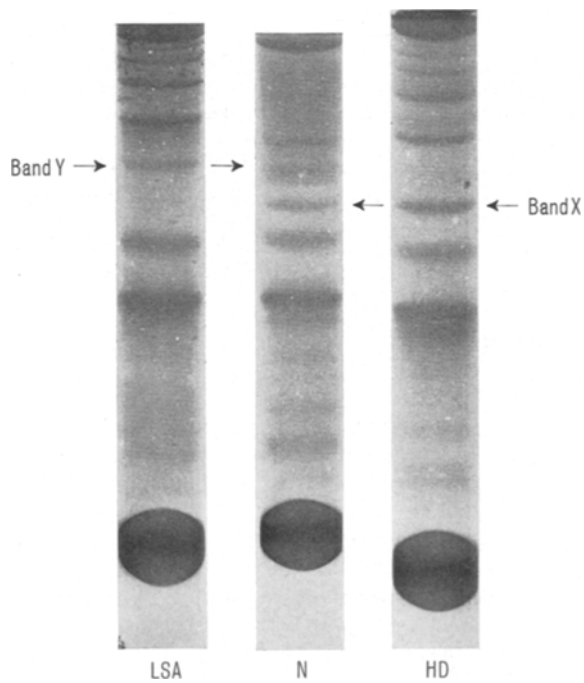
Discussion. 3 explanations are possible for the absence of band X or Y in certain sera: (a) the absence of synthesis; (b) partial synthesis of the 2 protein bands X and Y; or (c) changes in the structure of X or Y, causing the bands to occupy a new position in the gel patterns.

Preparative disc electrophoresis to isolate X and Y from control sera may resolve the nature of the defects. The technique[3] of preparative disc electrophoresis utilizes a long migration path (22 cm); thus, the 2 proteins could be obtained in reasonably pure form. Antiserum prepared against X or Y could then be used to test for homologous antigen in patient whole sera. Immunologic assay is sensitive and might detect microgram quantities of either protein, depending on antigenicity. Structurally modified X and Y should be revealed by their cross-reactivity. The location of these proteins will be pinpointed by immunodiffusion of disc resolved samples[4], provided they do migrate in polyacrylamide gel[5].

Normal serum proteins missing in patients with hematologic neoplasms

Patient group[a]	Total	No. band X	No. band Y
LSA	28	5	12
CGL	12	4	3
CLL	11	3	2
L	18	3	10
GFL	2	1	1
MM	5		1
AML	3	0	2
HD	18	0	13

[a] LSA, lymphosarcoma; CLL, chronic lymphocytic leukemia; CGL, chronic granulocytic leukemia; L, undifferentiated lymphoma; MM, multiple myeloma; HD, Hodgkin's disease; AML, acute myeloblastic leukemia; GFL, giant follicular lymphoma.



Polyacrylamide disc electrophoresis of human serum stained with Coomassie brilliant blue R-250. Normal (N) sample shows band X, which is missing from the lymphosarcoma (LSA) sample, and band Y, missing from the Hodgkin's disease (HD) sample.

Résumé. La méthode d'électrophorèse en gel avec coloration spéciale est utilisée ici pour la comparaison des protéines sériques de 97 malades porteurs de néoplasme avec celles de 33 contrôles normaux. Deux bandes, présentes dans tous les contrôles représentant aux moins deux différentes protéines avec une mobilité d'α-2-globuline, sont absentes dans 60 sérums de malades.

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