thyroiditis. Sometimes we found in mice and rats with this disease a spreading of the lymphocytes from the aberrant thymus tissue into adjacent thyroid tissue, but this may well be an artifact.

The thymic tissue in the thyroid gland reflects the close embryological evolution of these 2 glands. The aberrant thymus tissue does not seem to be derived from the thyroid. It looks quite normal and we have no reason to believe that the aberrant thymus tissue in the thyroid is not functioning. It was shown that aberrant parathyroid tissue in the neck and thymus in rats is normally functioning and can explain why many parathyroidectomized rats do not develop hypocalcemia[7]. Therefore, histological examination of the thyroid area is important in all thymectomized animals, in order to exclude the possibility of additional thymus tissue[8].

Résumé. Dans différentes lignées de souris et de rats, on a souvent trouvé du tissu thymique d'aspect normal au voisinage ou dans la thyroide même. Les animaux thymectomisés doivent être examinés pour contrôler la présence d'un tissu thymique aberrant.

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∅-Isoantigenic Marker in Phytohemagglutinin-Responding Mouse Blood Lymphocytes

Studies based on thymectomy and/or bursectomy[1,2] and on thymus/bone marrow grafting in karyologically distinguishable syngeneic strain combinations[3-5] indicate that the proliferative response induced in vitro by phytohemagglutinin (PHA) and some other mitogenic agents is under thymic control, and that the cells participating in the response are predominantly T-(thymus dependent) lymphocytes. The introduction of the ∅-isoantigen[6], as a marker for thymus-dependent lymphocyte in mouse[7,8], has made it possible to analyze the participation of T-cells in immune responses by using an alternative experimental design. In this communication we wish to report that depletion of ∅-bearing cells from mouse blood lymphocyte population abolishes the PHA-response, and that all but a minor fraction of the blast cells transformed by PHA in culture contain the ∅-antigen on their surface.

Materials and methods. Blood was collected from adult male CBA/T_6T_6 mice, which received intravenously 0.15 ml of 1:3 diluted *B. pertussis* culture eluate 3 days prior to the bleeding. The pertussis treatment, which was done to increase the cell yield[9,10], slightly decreased the number of ∅-positive cells in the blood (Table I). Optimum conditions for culture have been described before[10,11]. Blast cell counts and uptake of 3H-thymidine (New England Nuclear Corporation, Boston, Mass, – Sp. act. 6.7 mCi/m*M*) were used for the evaluation of the response[10]. ∅-isoantiserum was produced in AKR/Jax mice by 5 weekly injections of CBA/Jax thymus cells as described by RAFF[7,12]. Fresh hamster and guinea-pig sera were used as sources of complement[12]. The cytotoxic titer of the antiserum against CBA/T_6T_6 and C3H/HeJ thymus cells was 1:64–128. Normal AKR/Jax serum was used as control. The cytotoxicity tests were performed according to RAFF[12] at 1:2 and 1:4 dilutions of antiserum using 2 different methods: by the trypan blue dye exclusion test with purified lymphocytes populations, or by preparing cell smears after treatment with antiserum and complement using a Shandon cytocentrifuge (Shandon Scientific Co. Ltd., London NW 10)[13]. The latter of the 2 methods also permitted morphological examination of surviving cells. In cases where lymphocytes were cultured after the antiserum and complement treatment, the sera were sterilized by Millipore filtration (Millipore Filter Corporation, Medford, Mass. – pore size 0.22 μm), and the cell densities were readjusted prior to culture.

Results and discussion. The elimination of ∅-bearing cells prior to the culture nearly completely abolished the PHA-response (Table II). These cultures, when harvested on the 3rd day, contained primarily small lymphocytes, usually in aggregates around macrophages, some eosinophilic granulocytes and few degenerating cells with distorted morphology (Figure). The occasional blast cells seen in these cultures were within the aggregates and

Table I. Cytotoxic effect of anti-∅ and complement on mouse blood lymphocytes before and after *B. Pertussis* treatment[a]

Treatment	Dead cells (%) Blood lymphocytes (no pertussis)	Blood lymphocytes (pertussis)	Thymus cells
None	2	6	9
Anti-∅ + C′	78	70	94
AKR serum + C′	5	4	8

[a] Target cell population purified from red cells and granulocytes by NH_4Cl lysis and short term incubation on plastic. Antiserum dilution 1:4. Trypan blue (0.1%) dye exclusion test.

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nearly all of them showed a somewhat different morphology with a coarser nucleus and less basophilic cytoplasm as compared to the blasts seen ordinarily after PHA-response.

When ordinary PHA stimulated cultures were harvested on the 3rd day, and the cells treated with anti-ø and complement, most of the blast cells and the majority of the lymphocytes were lysed, whereas macrophages and eosinophils were remarkably resistant to lysis (Table III, Figure). There was, however, in every experiment, a small but a constant number of blast cells, ranging from 4–6% of the total number of blasts, which were not lysed by the treatment.

Since a fraction of blast cells in PHA-stimulated cultures were seemingly resistant to lysis by ø-antiserum and complement, 3 possibilities concerning the nature of these cells were available: that these cells were B-blasts

Table II. *PHA*-response after elimination of ø-bearing cells prior to culture

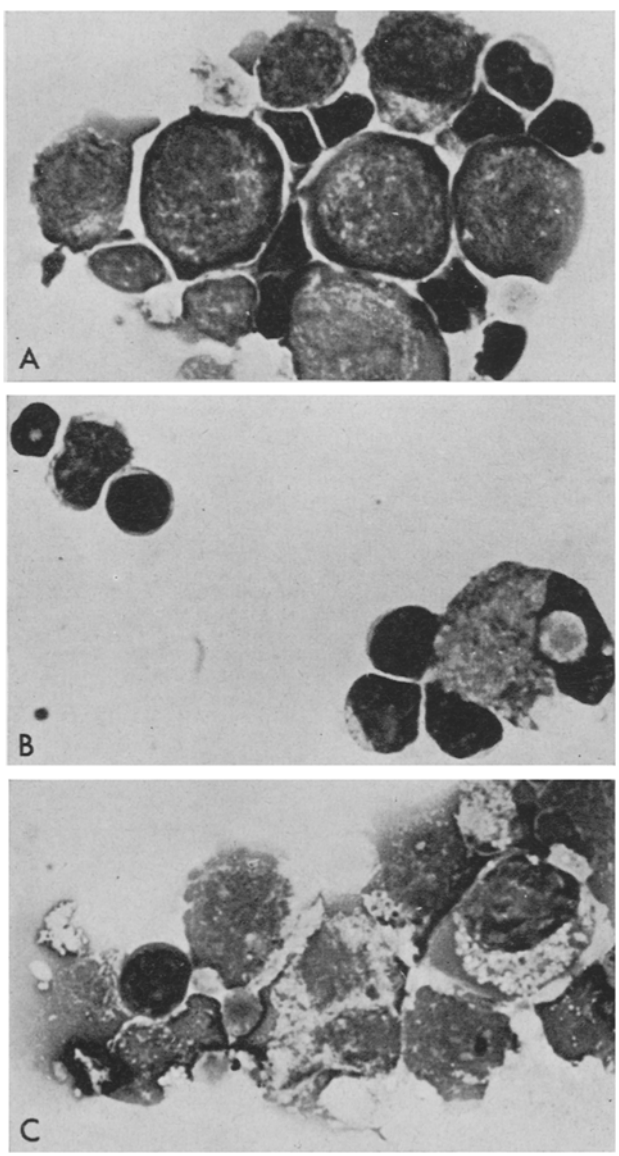
Treatment	Evaluation of response[a]	PHA present	PHA absent
None	Blasts (%)	75.1	4.3
	Labelled cells (%)	37.8	3.6
	cpm/culture	7990	210
Anti-ø + C′	Blasts (%)	2.8	1.0
	Labelled cells (%)	2.3	1.0
	cpm/culture	180	45
AKR-serum + C′	Blasts (%)	71.4	4.2
	Labelled cells	38.8	3.0
	cpm/culture	7550	190

[a] Evaluation of the response at 3rd day in culture. Blast cell counts performed from May-Grünewald-Giemsa stained smears. Values represent the mean of triplicate determinations. For technical details, see ref.[13].

Table III. Effect of anti-ø and complement on *PHA* stimulated mouse blood cells at 3rd day in culture

Treatment	Viable cells (%)[a]					Dead cells %
	Blasts	Lympho-cytes	Eosino-phils	Macro-phages	Unclas-sified	
None	66.5	12.5	1.0	2.5	0.5	17.0
PBS + C′	56.5	14.5	3.5	6.0	0.5	18.0
Anti-ø + C′	2.5	2.5	4.5	12.0	4.0	74.5
AKR-serum + C′	55.5	11.5	4.0	7.0	2.5	19.5

[a] Cell counts made from May-Grünewald-Giemsa stained smears. PBS = phosphate buffered saline.



Effect of ø-antiserum and complement on PHA-response: A) Normal response. B) Effect of treatment at day 0; blast transformation is inhibited. C) Effect on day 3; the majority of blasts and small lymphocytes are lysed; 1 surviving lymphocyte and 1 macrophage present in the field.

(blasts deriving from non-thymus processed lymphocytes) responding by antibody formation against some antigenic substances in the culture medium; that the resistant blasts were not lymphoid cells but maybe myeloid in their origin; or that these blasts were T-blasts possessing too few antigenic determinants on their surface to be lysed. Direct immunofluorescence with FITC-conjugated goat-antimouse-Ig (Hyland Laboratories, Los Angeles, Calif.)[14] of smears fixed in 96% ethanol failed to demonstrate immunoglobulin within their cytoplasm, although parallelly stained freshly prepared mouse spleen cell suspensions contained brightly positive cells. Stainings for naphtol-AS-*D*-chloracetate esterase[15] and with Sudan black B[16], known to be relatively specific to the myeloid series of cells, were negative in the resistant blasts but positive in myeloid cells of parallelly stained mouse bone marrow imprints. In the absence of any direct proof, the last of the possibilities, however, seemed to the most feasible to accept. This is because the amount of ø-antigen on peripheral lymphocyte surface apparently varies from cell to cell[17,18], and it is further known that the expression of

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histocompatibility antigens on cell surface is extremely variable within a rapidly dividing cell population[19, 20] as the PHA-induced blast population is at the peak of the response.

Note added in proof. Since this manuscript was submitted for publication, J. J. T. Qwen, P. Hunter and M. C. Raff (Transplantation *12*, 231 (1971)) have reported that the ∅-antigen is present in most blast cells resulting from stimulation with PHA in diffusion chambers in vivo.

Zusammenfassung. Lymphozyten, die in vitro auf das Phytohämagglutinin reagieren, tragen den ∅-isoantigenen Markierer auf ihrer Oberfläche. Die Abkömmlinge dieser Zellen sind zum allergrössten Teil ebenfalls ∅-positiv.

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'Halo Formation' as a Characteristic of the Interaction Between Ragweed Pollen and Saliva

For the primary purpose of discerning differences in the handling or treatment of ragweed pollen between normal and allergic individuals, I initiated a study of the interaction between ragweed pollen and body constituents (fluids or tissues).

Initially, I chose to use saliva from both allergic and nonallergic individuals in our investigations because saliva is the only secretory fluid that could be interacting with airborne pollen and that could be obtained in large quantity without stimulation.

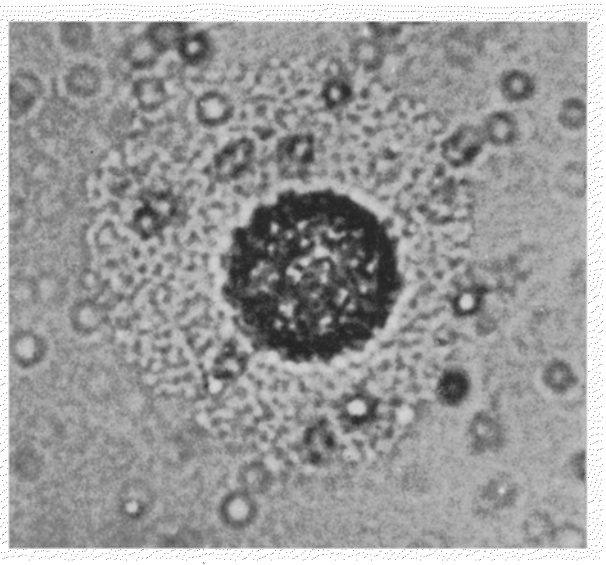
Saliva was collected without stimulation in a beaker that was immersed in ice and water, centrifuged cold, and stored at 0°C, but not frozen. The saliva donors consisted of 6 individuals who were allergic to the pollen and of 21 individuals who were not allergic to the pollen. No donor was showing allergic manifestations at the time of the saliva collections. Evidence of allergy consisted of clinical history of ragweed pollinosis and history of a positive skin test.

The ragweed pollen (*Ambrosia trifida*) was collected from cut plants by allowing it to fall onto aluminium foil. The pollen was left untreated and was stored in a vacuum dessicator to keep it dry and to avoid air oxidation.

In order to study any possible interactions between the pollen and the saliva, a drop of saliva was mixed with about 20 µg of fresh, mature ragweed pollen (about 800–1000 grains) on a glass slide; a coverslip was added and sealed on with Permount®. The slides were then incubated at room temperature for 3–14 days. These incubations were prepared with the 6 allergic and the 21 nonallergic saliva samples. As control media, we used distilled water, saline, and isotonic potassium chloride solution in place of the saliva. The incubation slides were examined intermittently under the microscope.

There were visible events common to all media. However, some of the pollen grains in the allergic saliva demonstrated a change that was manifested in the form of a halo of white, mat-like appearance around the pollen grain (Figure). Halos have been seen to grow in diameter as though emanating from the grain. If the slide is not disturbed, the halo will remain; if currents are developed, it washes away. The earliest appearance of halo formation has been noted at 18 h, but usually halos develop at about 24 h after the onset of incubation.

In 57 cultures of pollen and nonallergic saliva from 21 nonallergic individuals, only one culture showed any indication of the presence of halos; whereas halos were seen in 24 out of 29 cultures of pollen and allergic saliva from the 6 allergic individuals. Of the 5 negative results, 2 were obtained with the saliva of an individual who was on medication at the time of saliva collection. Usually, if one halo is seen on a slide, more can be found by careful examination of the slide. The incidence of halos on a slide has varied from several up to 30%.



Halo formation on a ragweed pollen grain that had been incubated in the saliva of an allergic individual. ×1300.

Zusammenfassung. Wenn Pollen von *Ambrosia trifida* mit dem Speichel von mit Allergie belasteten Personen zusammen kultiviert wird, so entsteht um die Pollenkörner ein Hof («halo»), was in Kulturen von nicht allergischen Personen nicht der Fall ist.

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