

Measurement of Lymphocyte Activation by a Chromatin Topo-optical Reaction

Mechanism and Specificity of the Test

J. M. Baló-Banga, L. Molnár, M. Nováki, and J. Leibinger

Department of Dermatology, Semmelweis University School of Medicine
(Director: Prof. I. Rácz, MD), Maria St. 41, H-1085 Budapest, Hungary

Summary. A rapid inexpensive method is presented for detecting peripheral blood lymphocyte chromatin activation by the neutral red “topo-optical” reaction, which causes strong and easily measurable birefringence in the lymphocyte nuclei. This reaction can be enhanced by fixing the cells with 150 mM/l NaCl in 70% ethanol and/or by treating the unfixed cellular suspensions with 0.2 M/l HCl to remove histones. In histone-removed preparations, 30 min DNase I treatment almost completely abolished the birefringent reaction, whereas RNase treatment resulted in only 18% loss.

Chromatin activation induced by enzyme inhibition increased chromatin birefringence significantly. The same phenomenon could be induced in sensitive subjects' lymphocytes by specific antigens or haptens much more rapidly. The monocytes were not activated to a significant extent. In non-sensitive subjects different kinetics of antigen or hapten-dependent activation and no cytotoxic effects have been observed. Depletion of T-lymphocytes in vivo in SLE patients or by in vitro treatment with 0.5 mM/l KCN as well as with 0.02% trypsin has caused a significant drop in the mean chromatin birefringence. The effect of trypsin was reversible.

Key words: T cell-dependent — Lymphocyte chromatin activation — Birefringence

Zusammenfassung. Eine relativ billige Schnellmethode für den Nachweis von Chromatin-Aktivierung in Blut-Lymphocyten mittels einer »topooptischen« Reaktion mit Neutralrot, die zu einer starken und leicht meßbaren Doppelbrechung im Zellkern führt, wird beschrieben.

Diese »topooptische« Reaktion wird durch Fixierung der Zellen mit 150 mM/l NaCl in 70% Alkohol bzw. durch Behandlung der nicht-fixierten Zellsuspensionen mit 0,2 M/l HCl für die Entfernung der Histone verstärkt. In histonfreien Präparaten wird durch eine 30-minütige DNase I-Behandlung die

Doppelbrechung nahezu vollkommen zum Verschwinden gebracht, während RNase nur 18% Verminderung herbeiführt.

Chromatin-Aktivierung, die durch Enzym-Hemmung induziert wurde, führt zu einer signifikanten Erhöhung der Chromatin-Doppelbrechung. Dasselbe Phänomen ließ sich in den Lymphocyten sensitiver Personen mittels spezifischer Antigene oder Haptene wesentlich schneller erzielen. Die Monocyten zeigten keine signifikante Aktivierung. In nicht-sensibilisierten Probanden führte die Inkubation mit Antigenen oder Haptenen zu einer, mit differenter Kinetik gekennzeichneten Aktivierung, wobei keine Zeichen von Cytotoxizität vorhanden waren. Die Depletierung der T-Lymphocyten *in vivo* bei SLE Patienten oder mittels *in vitro* Behandlung von 0,5 mM/l Lösung von KCN oder von 0,02% Trypsin führte zu einer signifikanten Abnahme der durchschnittlichen Chromatin-Doppelbrechung. Die Trypsinwirkung war reversibel.

Schlüsselwörter: T-Zellen-abhängig – Lymphocyten Chromatinaktivierung – Doppelbrechung

Chromatin activation is a process which progresses in the interphase nuclei upon cell stimulation. Active cytogenetic units appear as demonstrated by the electron microscope (Klug 1978). Acetylation of the nucleosomal histones occurs at well defined positions (Allfrey et al. 1973; Bradbury 1978). A similar process can be induced by butyrate which in turn suppresses histone deacetylation and leads to an accumulation of multiacetylated forms of histones H₃ and H₄ with an increased DNase I sensitivity of the associated DNA sequences (Vidali et al. 1978).

A Swedish group previously used fluorochromes and found that 1–60 min after phytohemagglutinin (PHA) stimulation the acridin orange (AO)-stained leukocytes exhibited a pronounced increase in fluorescence intensity (Killander and Rigler 1965). The average increase in the number of AO binding sites was 2-fold after PHA stimulation (Killander and Rigler 1969); the possibility of staining artifacts could be excluded. This was the first indication of immunologically induced chromatin activation.

An important methodological improvement, by which the expensive fluorescence microphotometry can successfully be displaced, has been made by Surján and Sebók (1973) and Baló-Banga and Molnár (1976). This improvement is based on a neutral red topo-optical reaction and subsequent birefringence measurement of the cellular chromatin. This method yields permanent preparations and makes measurement relatively simple by using a comparatively cheap plate compensator instead of a microspectrofluorimeter.

The optimal conditions for this reaction have been elaborated. The first application in dermatology was introduced by Baló-Banga et al. (1978a–c) who measured peripheral blood lymphocyte chromatin activation by a potent skin sensitizier, viz., 2,4-dinitrochlorbenzene (DNCB). Moreover, attempts were made to correlate the chromatin activation with known antigen responses (Baló-Banga and Molnár 1976; Baló-Banga et al. 1978a; Király et al. 1979) and with experimental modifications of impaired immune response to PHA and DNCB by Levamisole (Baló-Banga et al. 1978c).

On this basis the test was successfully adapted and used to detect an aminopyrin allergy in a cured Lyell syndrome patient (Krámer et al. 1978).

In the present paper we intend to describe the final version of the lymphocyte chromatin activation technique which has been elaborated for dermatological purposes. We also wish to present some experimentally supporting clues which might lead to a better understanding of its molecular mechanism.

Case Report

Altogether 19 cases were investigated. Three in-patients of the Dermatological Clinic, Budapest, with artificially induced DNCB sensitivity (Catalona et al. 1972; Hamar 1975), were studied to test cellular immune reactions. The patients had shown no hypersensitivity to tuberculin (PPD) and reacted with eczematous eruption upon reexposure to DNCB. One patient with alopecia totalis, who had been sensitized with DNCB, developed a hyperergic bullous skin reaction. Another patient was admitted with erythema multiforme due to penicillin inhalation. His penicillin allergy was proven later. He had no contact with DNCB. Three cases of tuberculoallergy reacted with a hyperergic erythematopapulous skin reaction to PPD. These patients were not in contact with DNCB. One healthy subject, a laboratory technician, had a former history of aminopyrin sensitivity. Three out-patients with no immunological abnormalities who were healthy, except for a mild case of psoriasis, provided control cell suspensions for DNase, RNase, trypsin, and KCN studies. Six severely ill in-patients with systemic lupus erythematosus (SLE) were also studied after they had been diagnosed according to ARA¹ criteria (Decker et al. 1975).

Material and Methods

Desoxyribonuclease I (DNase, Lot 62791) was obtained from Calbiochem, Los Angeles (USA). Ribonuclease (RNase, 550 µg/ml stock solution, heated at 90° C for 10 min to destroy DNase activity) was a generous gift from Dr. H. Altmann, Seibersdorf (Austria). Trypsin, free of chymotrypsin (Lot 53955), was the product of Calbiochem and applied in a solution of 0.16 mg per 10⁶ living cells. Pure aminopyrin was kindly placed at our disposal by the Central Pharmaceutical Stores of the Semmelweis University, Budapest (Hungary). All other chemicals were of the highest available purity.

Cell Separation and Processing; T-cell Typification

The isolation of the lymphocyte fraction from the peripheral blood was carried out by the "Lymphoprep" centrifugation technique of Wottawa et al. (1974) as adapted for our laboratory by Molnár et al. (1978). The isolated cells were washed twice with medium TC 199 and resuspended finally with the same medium buffered by 50 mM/l Tris-HCl. The final pH was 7.2–7.4. The T-lymphocytes were determined according to Jondal et al. (1972) with minor modifications as suggested by Fekete et al. (1974). One hundred microliters of the lymphocyte suspension containing 5 × 10⁶ cells/ml was mixed with 150 µl of inactivated calf serum and 100 µl of washed 2% sheep erythrocyte suspension, and the mixture was centrifuged at 400 × g for 5 min. After 21 h at 4° C the number of spontaneous rosette-forming elements was determined under the microscope. At least 200 cells were counted. T-lymphocytes bound three or more erythrocytes on their surfaces. Normal values were 70–75%.

In experiments with KCN and trypsin the lymphocyte suspensions were incubated with the given amounts of these substances for 0, 15, 30, 45, and 60 min, respectively, at 37° C. Samples for spontaneous rosette formation and for chromatin birefringence measurements were taken separately from these incubation mixtures at the given times. Direct measurement of chromatin birefringence in rosette-forming cells was unsatisfactory because of the polarization quenching effect of the sheep erythrocytes.

Chromatin Birefringence Measurements

The isolated 95–100% viable lymphocytes, measured by the trypan blue exclusion test, were diluted to a cell density of 1.1 × 10⁶ cells/ml. The incubation with the proper amounts of antigens and haptens was carried out by dissolving these substances in one tenth of the cell volume. The length of incubation was

1 American Rheumatological Association

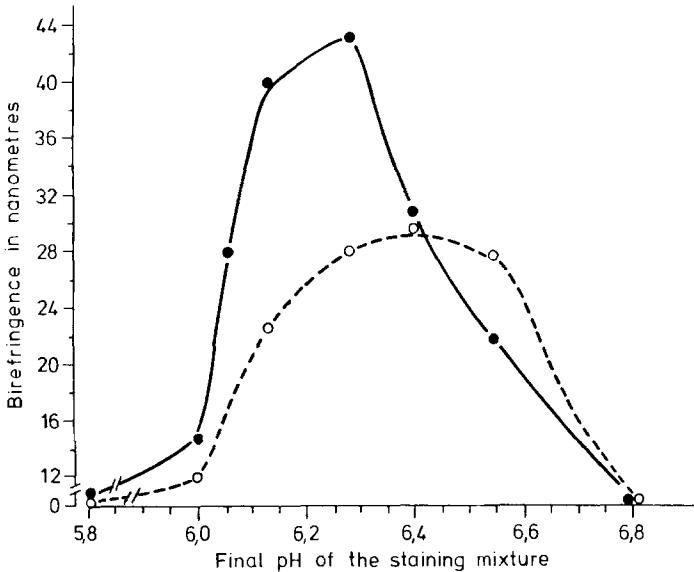


Fig. 1. pH dependence of the chromatin-neutral red topo-optical reaction. The lymphocytes of a DNCB sensitive subject were incubated with 500 nM/l DNCB for 20 min. Salt-ethanol fixed preparations were stained by 0.04% neutral red in 105 mM/l of NaCl, 50 mM/l of phosphate buffer for 7 min, rinsed and post-stained by 2% K-ferricyanide for 2 min at different pH values. ○-----○ Control samples, incubated with 3% DMSO⁺, the solvent for DNCB (⁺ dimethyl-sulfoxide). ●-----● DNCB incubated samples

20 or 30 min at 37°C except for the time course experiments. Then the incubation mixtures were immersed into crushed ice and samples were sedimented, fixed, and stained as described earlier (Baló-Banga and Molnár 1976; Baló-Banga et al. 1978b). Finally, the preparations were measured with an Amplival Pol D type microscope of Zeiss (Jena, GDR), equipped with a $\lambda/8$ rotary compensator. The average angles of compensation in both directions of at least 30 cells selected at random in each preparation were used for calculating retardation of birefringence in nanometers (nm) for each individual cell separately with Brace's equation (25). These readings were used to calculate the mean values and standard errors characteristic of a given preparation.

Results

Molecular Mechanisms Underlying the Chromatin's Topo-optical Reaction with Neutral Red

There is a basic difference due to the conditions under which neutral red is applied to a cellular preparation. In non-fixed smears the plasma membranes and presumably the lysosomes bind the dye which results in an extremely low grade of birefringence. Fixation by 70% ethanol changes the topography of the birefringence within the cell (Table 3) and transfers the neutral red topo-optical reaction into the nuclei. 150 mM/l NaCl in 70% ethanol has been found to be optimal in the fixative (Table 3). 300–500 mM/l NaCl depresses birefringence to the level of only 70% ethanol-fixed samples (results not shown in details).

The reaction between chromatin and neutral red results in birefringence only if the pH of the staining mixture is between 6.0 and 6.6. Moreover, in subjects who

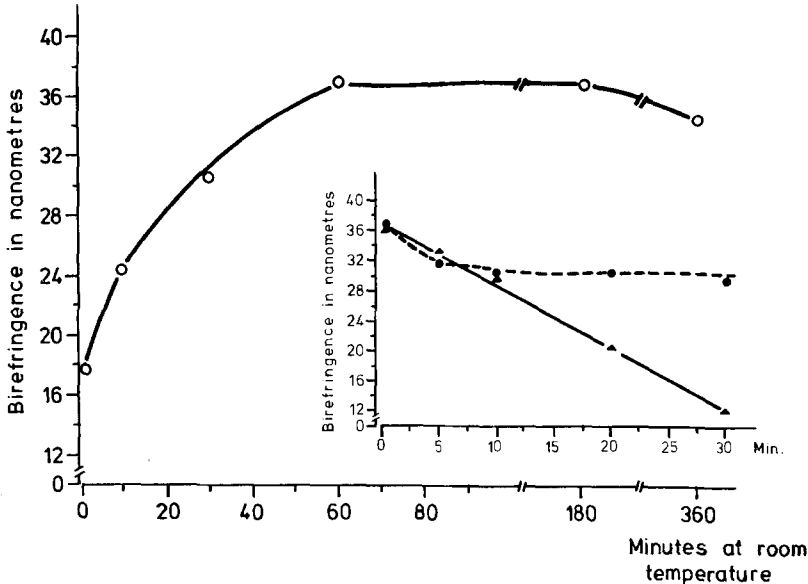


Fig. 2. The effects of 0.2 M/l HCl, DNase and RNase, at 25°C. 3.0×10^5 cells, sedimented onto microscopic slides in a 1 cm \varnothing circle were treated by 0.2 M/l HCl (○—○). Samples, treated by 0.2 M/l HCl for 75 min were subjected to 30 μ g of DNase I (sp. act. 2 I.U./ μ g), dissolved in 5 mM/l $MgCl_2$; 100 mM/l acetate buffer at pH 5.0 (▲—▲). Samples, treated by 0.2 M/l HCl for 75 min were subjected to 30 μ g of RNase, dissolved in 100 mM/l NaCl at pH 7 (●—●)

had artificially been sensitized to DNCB we found the rate at which birefringence increased to also depend on the pH of the staining mixture (Fig. 1) in the stimulated samples. It reached its peak value at pH 6.27. This value seemed relatively more important in stimulated than in non-stimulated samples. The latter have a broad plateau between pH 6.27 and 6.58. The NaCl concentration was found optimal if 105 mM/l were present in the staining buffer containing neutral red. Potassium ferricyanide in the final step forms a stable insoluble complex with the already structure-bound neutral red. This has a double purpose: (a) it blocks any removal of bound neutral red and so contributes to permanent birefringent staining of the preparations; (b) it modifies the self-absorption of polarized light that is emitted by a tungsten or a quartz iodine lamp so that the efficiency of the measurement increases.

To answer the basic question, i.e., which of the chromatin constituents would primarily bind neutral red and thus account for birefringence, a set of experiments was performed to remove histones by 0.2 M/l HCl treatment and to subsequently assay chromatin-neutral red-birefringence in the usual way. As shown in Fig. 2, even unfixed preparations were strongly birefringent upon histone removal in as short as 1 h and displayed a plateau between the 1st and 3rd h of treatment which later slowly declined. In histone-removed preparations 30 min of DNase treatment reduced birefringence to a minimal value of 12.1 ± 1 nm. The time course of DNase treatment was linear. RNase produced approximately a 20% drop in birefringence within 10 min with no further reduction up to 30 min of treatment. The effects of DNase and RNase are shown in the inset of Fig. 2.

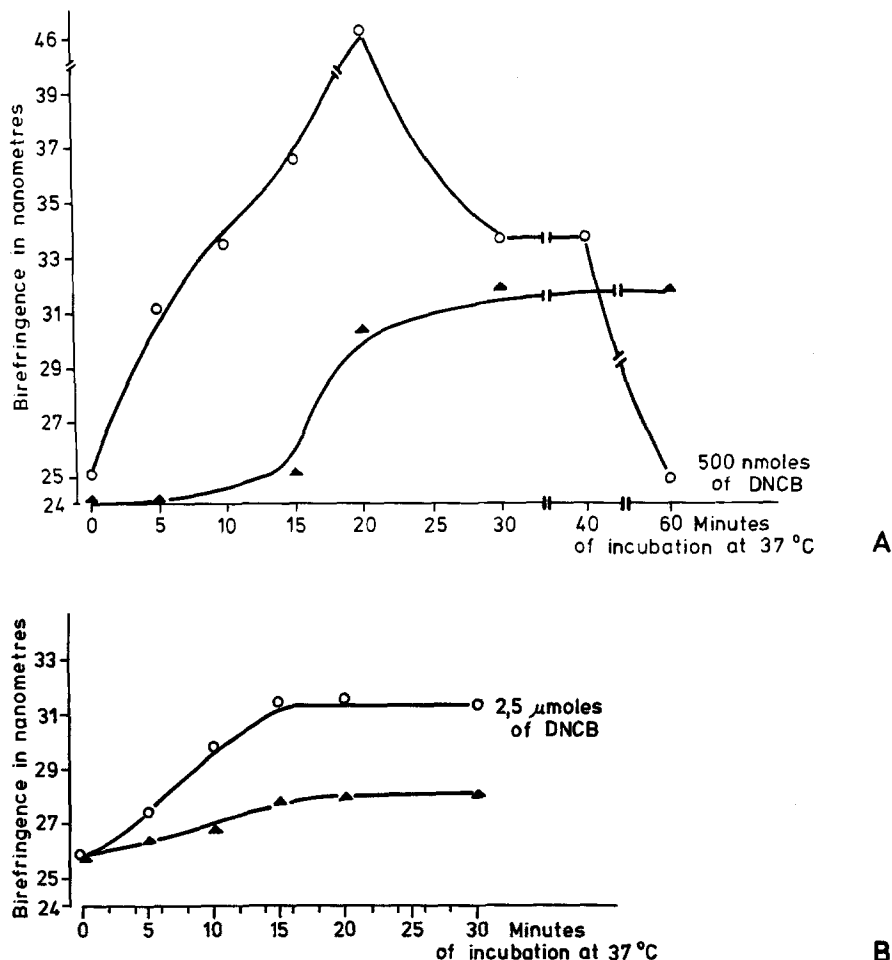


Fig. 3. A Time courses of the chromatin activation in a DNCB sensitized and B non-sensitized patient at 37°C. ○—○ DNCB, dissolved in 3% DMSO. ▲—▲ Control, 3% DMSO

Increase of Intranuclear Birefringence Upon Butyrate Suppression of Histone Deacetylation

Incubation of the lymphocyte suspensions in the presence of 6 mM/l final concentration of butyrate led to a significant increase of chromatin birefringence within 90 min (results not shown in detail).

Time Course of Chromatin Activation Reaction of Sensitized and Non-sensitized Humans

The time dependence of the increase in chromatin birefringence is shown in Fig. 3. Figure 3A summarizes the hapten-induced chromatin activation of a DNCB-sensitized man who had developed a bullous skin reaction upon reexposure to this compound. The reaction stopped to proceed after 20 min of incubation and was

Table 1. Specificity of the chromatin activation reaction among the isolated white blood cells. The cells of an aminopyrin-sensitive person were incubated with and without the drug for 20 min at 37° C

Cell types	Incubated with	
	3% DMSO solvent	500 nM/l aminopyrin
Mean birefringence in nm ± S.E.		
Lymphocytes	24.7 ± 1.0	37.4 ± 1.7
Monocytes	22.7 ± 0.8	25.4 ± 0.9

Table 2. Specificity of the lymphocytes' chromatin activation to the sensitizing substance. Each group consisted of three cases. The difference between DNCB and tuberculin (PPD) reactivity of the tuberculin sensitive cases was statistically significant ($P = 0.0384$). Incubation with the substances was carried out for 30 min at 37° C

Origin of cells	Exposing substance	DNCB-sensitive cases	Tuberculin-sensitive cases
		Mean birefringence in nm ± S.E.	
500 nM/l of DNCB		35.1 ± 1.0	21.5 ± 4.5
1 µg PPD ^a per 10 ⁶ cells		22.8 ± 5.2	32.4 ± 0.8

^a With 43% protein content

followed by a strong decrease in birefringence accompanied by visible signs of chromatin damage at 40 and 60 min. The dynamics of chromatin birefringence is shown in Fig. 4 (A, B, C, D). The DNCB was present in 500 nM/l concentration. Another type of response to DNCB is shown in Fig. 3B, where a person's lymphocytes had no previous contact with this compound. Two point 5 µM/l of the hapten were necessary to induce a smaller increase in chromatin birefringence with no demonstrable signs of chromatin damage and a plateau between 15 and 30 min.

The Specificity of the Immunologically Induced Chromatin Activation Reaction. Effects of Cellular Damage

The lymphocyte seems to be the main cell type that responds to antigens and haptens by increased chromatin birefringence. Table 1 compares the major cell types present in the isolated white blood cell fraction, with respect to their responsiveness to a specific sensitizer, aminopyrin, in a clinical case of proven allergy to this drug. Monocytes were easily distinguished in the preparations. In Table 2, the data show that only the sensitizing substance induces lymphocyte chromatin activation. Finally, Table 3 summarizes the conditions that may interfere with the process of chromatin activation. Lymphocyte suspensions are badly damaged by freezing and thawing with concomitant increase of trypan blue staining and loss of viability from 95 to 0.1%. This resulted in an almost complete abolition

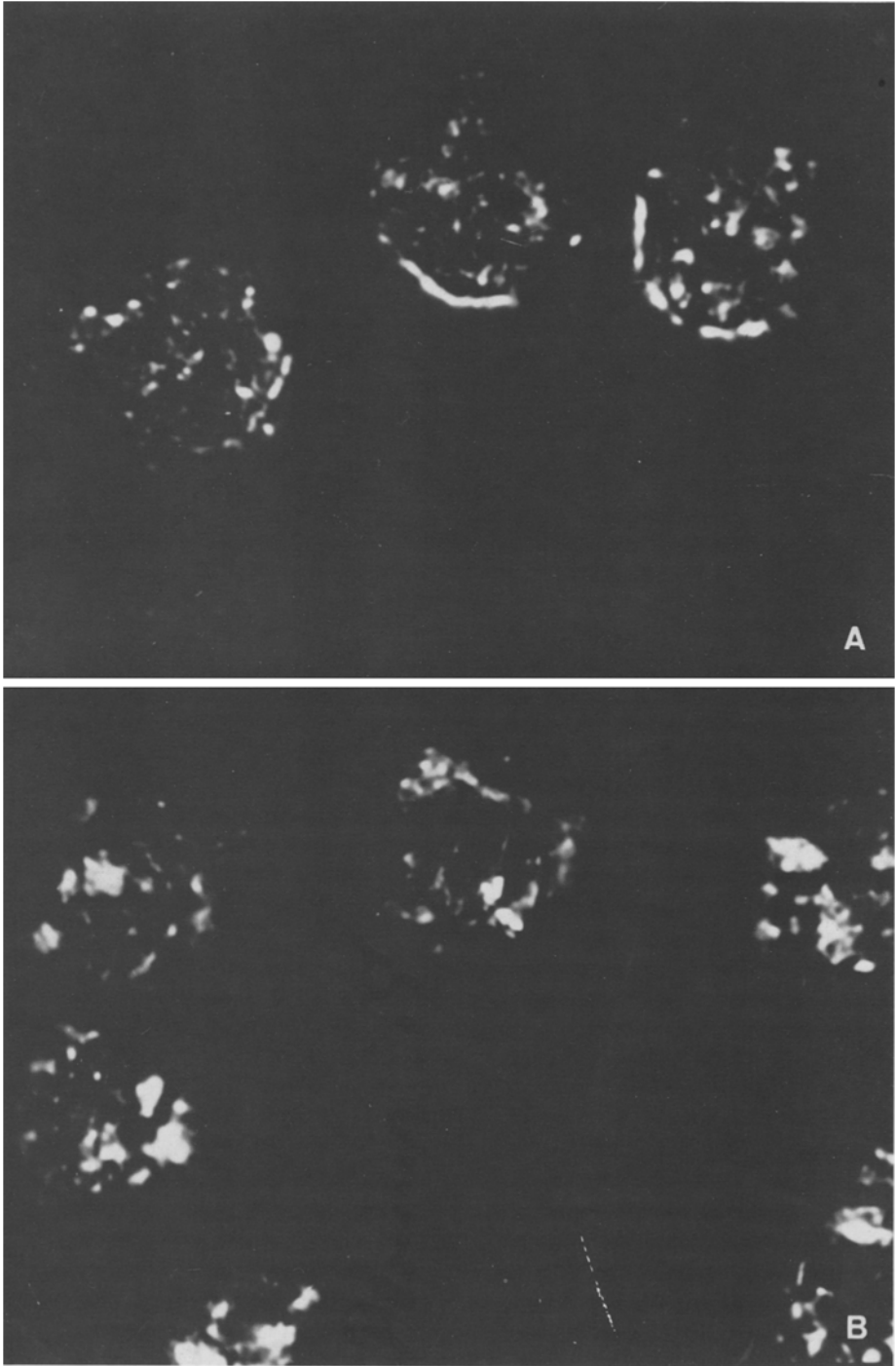


Fig. 4A—C. Characteristic chromatin morphology in polarized light. The lymphocytes from a DNCB-sensitive patient were incubated at 37° C with 500 nM/l concentration of the sensitizing hapten. **A** 5 min, **B** 15 min, **C** 20 min, **D** 40 min. The photomicrographs were taken in the perpendicular position of the analyzer and polarizer (Opton photomicroscope, Planachromat objective; magnification: $\times 1,500$)

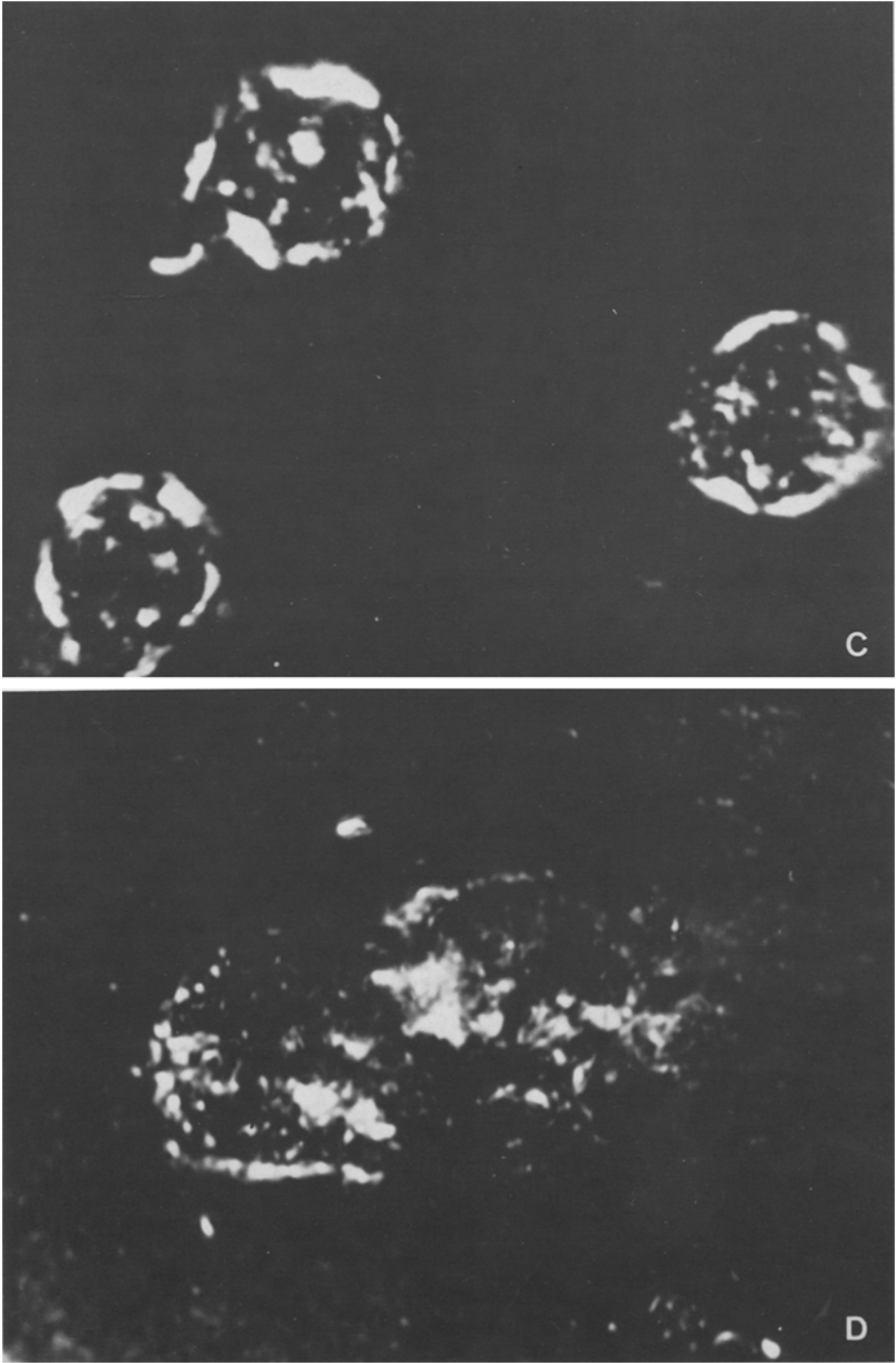


Fig. 4C, D

Table 3. Changes in the lymphocyte chromatin birefringence due to the experimental conditions

Preparations	Mean birefringence in nm \pm S.E.	T-lymphocytes %
1. Sedimented, unfixed cells	17.9 \pm 0.4	68
2. Sedimented cells, fixed by 70% ethanol	19.5 \pm 1.0	70
3. Sedimented cells, fixed by 150 mM/l NaCl in 70% ethanol	28.4 \pm 1.1 (of 16 independent experiments)	69.8 \pm 1.8 (of six indepen- dent experiments)
4. Frozen and thawed cellular suspension (fixed as it appears in line 3)	12.5 \pm 1.2	0.2
5. Cellular suspension, incubated in the pre- sence of 0.5 mM/l KCN for 80 min; (fixed as it appears in line 3)	14.9 \pm 0.9 (of two independent experiments)	5.0
6. Stimulated lymphocyte suspension, incu- bated with 0.16 mg/ml 1×10^6 cells trypsin for 60 min (fixed as it appears in line 3)	17.8 \pm 0.6 (of two independent experiments)	4.0
7. Non-stimulated lymphocyte suspension, incubated with 0.16 mg/ml 1×10^6 cells trypsin for 60 min; (fixed as it appears in line 3)	12.3 \pm 1.0 (of two independent experiments)	5.0
8. Lymphocyte suspensions from SLE pa- tients (fixed as it appears in line 3)	20.1 \pm 2.8 (of six independent experiments)	34.8 \pm 1.6

of chromatin birefringence. The average value was nearly as low as in DNase treatment (12.5 \pm 1.2 nm).

The incubation of the cells with 0.5 mM/l KCN for 80 min did not significantly affect trypan blue exclusion. There was, however, a gradual reduction in the T-cell count as witnessed by a 50 to 5% drop in the ratio of spontaneous rosette-forming cells (results not shown in full detail). Cyanide treatment, which was immediately washed out while the cells were cooled, only slightly affected rosette formation of the controls. The untreated samples showed only 15% more T cells (65% vs. 50%). With the decrease of T-markers, there was a marked gradual reduction in the average lymphocyte birefringence.

Mild trypsinization of the lymphocyte suspensions did not affect viability, but decreased the numbers of spontaneous rosette-forming elements exponentially. The average chromatin birefringence decreased in both stimulated and non-stimulated cellular suspensions in the same way. The coefficient of correlation between the gradual reduction of the T-cell counts and the mean birefringence values in stimulated samples was $r_s^2 = 0.9519$, whereas in non-stimulated ones $r_{ns}^2 = 0.9573$. On washing out trypsin after 60 min of incubation, we noted a rapid increase in the chromatin birefringence within 15 min. We observed a significant decrease of chromatin birefringence in six cases of SLE, where T-cell ratios ranged from 55 to 28% (average 34.8). Some correlation seemed to exist between the extent of in vivo T-depletion and the reduction of mean birefringence values.

Discussion

In spite of the fact that neutral red is a supravital dye, Ambrose and Gopal-Ayengar (1952) discovered that fixed nucleoprotein films could be stained by it, dissolved in 0.14 M/l salt solution and that such stained films exhibited visual dichroism in polarized light.

Data by Thoma and Koller (1977) have demonstrated that 100 mM/l NaCl and 50 mM/l sodium phosphate at pH 7 selectively liberated histone H₁ from chromatin in 30 min. H₁, unlike histones H₂A, H₂B, H₃ and H₄, is not necessary in the buildup of the nucleosome (Oudet et al. 1978), but is thought to affect the higher order DNA coiling in chromatin (Worcel and Benyajati 1977). Their conditions were similar to ours. Therefore, it is tempting to speculate that in our salt-ethanol treated and fixed preparations H₁ is removed from chromatin. Our experiments supplied evidence as to the site of neutral red binding in chromatin. DNA ought to be the main target, for only DNase and not RNase abolished the visual dichroism. Removal of all histones by 0.2 M/l HCl according to Cowden and Curtis (1973) has increased the birefringence by a factor of 2.

There is evidence that DNase primarily attacks internucleosomal (spacer) DNA (Worcel and Benyajati 1977), and leaves the basic units behind after it has decomposed the chromatin structure.

The fact that butyrate, a known inhibitor of histone deacetylation (Vidali et al. 1978), increased chromatin birefringence within 90 min, supplied indirect evidence that our method really measured chromatin activation.

The immunological triggering of the lymphocyte chromatin by extremely low amounts of a wide variety of sensitizing substances, among them drugs, occurred more rapidly than the enzymatic one. It displayed a bell-shaped dose response curve (Baló-Banga et al. 1980) and showed specificity to the sensitizing substance (Table 2). Structural differences among T-, B-cell, and macrophage chromatin have arisen from a computer analysis of digitized data, obtained by scanning microphotometry (Durie et al. 1978). We also found (Tables 1 and 3) that the chromatin in T-cells must be the important factor which determines the average birefringence values of the preparations. The partial T-depletion in SLE cases (e.g., Hara et al. 1975) was accompanied by a marked decrease in the mean chromatin birefringence; whereas an even more pronounced T-marker loss, due to the treatment with KCN or trypsin, reduced the birefringence almost to the levels of DNase-treated samples (Table 3). The effect of trypsin was completely reversible which is an argument against cell death. It is worthy to note that cell surface trypsinization which is known to remove cell-surface antigenic determinants (e.g., Fakhri and Tan 1975) did not abolish the differences between the mean birefringence of the previously stimulated and the control non-stimulated samples. This might explain why the average birefringence values of SLE-deriving cellular suspensions did not correlate better with the actual T-cell counts. The extent to which an *in vivo* stimulation takes place cannot be calculated. We conclude, therefore, that lymphocyte chromatin activation by antigens or haptens is a sensitive, rapid, and specific procedure which in viable lymphocytes primarily depends on the function of T cells. Its application seems to be suitable in detecting drug allergy (Baló-Banga et al. 1980).

References

- Allfrey VG, Inoue A, Karn J et al. (1973) Phosphorylation of DNA-binding nuclear acidic proteins and gene activation in the HeLa cell cycle. *Cold Spring Harb Symp Quant Biol* 38: 785–801
- Ambrose EJ, Gopal-Ayengar AR (1952) Molecular organization in giant chromosomes. *Nature* 169: 652–653
- Baló-Banga JM, Molnár L (1976) Lymphocyte chromatin activation and its interrelation with DNA damage. In: Altmann H (ed) *DNA repair and late effects*. Roetzer, Eisenstadt, pp 51–71
- Baló-Banga JM, Molnár L, Leibinger J (1978a) Biochemical analysis of the alterations during chromatin activation of isolated human lymphocytes. Congress report of the joint Meeting of the Hungarian Biophysical, Biochemical, and Physiological Societies, Pécs, 1977. *Acta Physiol Acad Sci (Hung)* 52: 198
- Baló-Banga JM, Molnár L, Nováki M (1978b) Messung der Chromatin-Aktivierung in den Lymphozyten mit topooptischer Methode. *Kisérlet Orvostud (Budap)* 30: 126–135
- Baló-Banga JM, Molnár L, Nováki M, Leibinger J (1980) Measurement of lymphocyte activation by a chromatin topooptical reaction. II. Application for detecting drug allergy. A clinical and experimental study. *Allerg Immunol (Leipz)* 26: 137–153
- Baló-Banga JM, Molnár L, Rácz I, Leibinger J (1978c) The effect of in vivo Levamisole treatment on the chromatin birefringence of systemic lupus erythematosus patients' lymphocytes with and without antigenic stimulation. Paper read at the VIIIth Meeting of the Hungarian Allergological Society. *Orv Hetil (Budap)* 119: 751
- Bradbury EM (1978) Histone interactions, histone modifications and chromatin structure. *Philos Trans R Soc Lond (Biol)* 283: 291–293
- Catalona WJ, Taylor PT, Robson AS, Chretien PB (1972) A method for dinitrochlorbenzene sensitization. A clinico-pathological study. *N Engl J Med* 286: 399–402
- Cowden RR, Curtis ShK (1973) Fluorescence cytochemical studies of chromosomes: quantitative applications of fluorescein mercuric acetate. In: Thaeer AA, Sernetz M (eds) *Fluorescence techniques in cell biology*. Springer, Berlin Heidelberg New York, pp 135–149
- Decker JL, Steinberg AD, Gershwin ME, Seaman WE, Klippel JH, Plotz PH, Paget SA (1975) Systemic lupus erythematosus. Contrasts and comparisons. *Ann Int Med* 82: 391–404
- Durie BGM, Vaught L, Chen YP, Olson GB, Salamon SE, Bartels PH (1978) Discrimination between human T- and B-lymphocytes and monocytes by computer analysis of digitized data from scanning microphotometry. I. Chromatin distribution patterns. *Blood* 51: 579–589
- Fakhri O, Tan RSH (1975) The effect of trypsin on cell surface antigens. *Cell Immunol* 15: 452–456
- Fekete B, Szegedi Gy, Gergely P, Szabó G, Petrányi Gy (1974) Technical problems of spontaneous rosette formation. A suggestion for standardization of the method. *Haematologia (Budap)* 8: 353–360
- Hamar M (1975) Investigations on the cellular immunity by dinitrochlorbenzene placed on the skin. *Orv Hetil (Budap)* 116: 2291–2293
- Hara M, Morimoto C, Takahashi H et al (1975) Peripheral blood T-lymphocytes during the course of systemic lupus erythematosus. *Keio J Med* 24: 263–273
- Jondal M, Holm G, Wigzell H (1972) Surface markers on human T- and B-lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with SRBC. *J Exp Med* 136: 207–215
- Killander D, Rigler R Jr (1965) Initial changes of desoxyribonucleoprotein and synthesis of nucleic acid in phytohaemagglutinin-stimulated human leukocytes in vitro. *Exp Cell Res* 39: 701–704
- Killander D, Rigler R (1969) Activation of desoxyribonucleoprotein in human leukocytes stimulated by PHA I. *Exp Cell Res* 54: 163–170
- Király K, Baló-Banga JM, Molnár L (1979) Examination of the immunocompetent cell composition of contact dermatitis. *Allerg Immunol (Leipz)* 25: 32–44
- Klug A (1978) Structure of chromatin. Introductory remarks. *Philos Trans R Soc (Lond) (Biol)* 283: 233–239
- Krámer M, Baló-Banga JM, Molnár L, Kovács I (1978) A case of cured Lyell syndrome. *Bőrgyógy Venerol Szle (Budap)* 54: 163–173
- Molnár L, Baló-Banga JM, Horváth A, Leibinger J, Ablonczy É, Nováki M (1978) Immune-complex phagocytosis by human polymorphonuclear granulocytes. *Acta Physiol Acad Sci (Hung)* 52: 33–39
- Oudet P, Germond JE, Bellard M, Spadafora C, Chambon P (1978) Nucleosome structure. *Philos Trans R Soc Lond (Biol)* 283: 241–258

- Surján L Jr, Sebók J (1973) Increase in intranuclear birefringence during chromatin activation reaction (preliminary note). *Exp Cell Res* 78:241–243
- Thoma F, Koller Th (1977) Influence of histone H₁ on chromatin structure. *Cell* 12:101–107
- Typisierte Kompensatoren für polarisationsoptische Untersuchungen. VEB Carl Zeiss Jena. Druckschrift No. 30-537-1:S 9
- Vidali G, Boffa LC, Bradbury EM, Allfrey VG (1978) Butyrate suppression of histone deacetylation leads to accumulation of multiacetylated forms of histones H₃ and H₄ and increased DNase I sensitivity of the associated DNA sequences. *Proc Natl Acad Sci USA* 75:2239–2243
- Worcel A, Benyajati Ch (1977) Higher order coiling of DNA in chromatin. *Cell* 12:83–100
- Wottawa A, Klein G, Altmann H (1974) Eine Methode zur Isolierung menschlicher und tierischer Lymphozyten mit Ficoll-Urographin. *Wien Klin Wochenschr* 86:161–163

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