Experimental contact sensitivity: a model for both antigen (hapten)-specific and innate immune mechanisms

M. Kataranovski, L. Kandolf Sekulović and Đ. Karadaglić

ABSTRACT

In the present study, experimental model of contact sensitivity to dinitrochlorobenzene (DNCB) in inbred AO rats was employed to determine both antigen (hapten)-specific as well as parameters of antigen-non-specific aspects of contact hypersensitivity (CHS) response. Following local epicutaneous application of 2% DNCB, increased spontaneous, hapten-stimulated and interleukin (IL)-2-driven draining lymph node cell (DLNC) proliferation was detected, reflecting the antigen (hapten)-specific aspect of CHS. Changes in the ratio of lymphocyte subsets were noted in DLNC in the sensitization phase of CHS. At the dose of DNCB employed for the elicitation of CHS in sensitized animals, increased activity of peripheral blood granulocytes (including activation, adhesion and cell survival) was detected. Collectively, these data demonstrate the activation of both antigen (hapten)-specific and innate immunity in contact hypersensitivity. The importance of studying both mechanisms in this same model has been discussed.

KEY WORDS

contact hypersensitivity, DNCB, lymphocytes, granulocytes, rat

Introduction

Skin responds to toxic agents by various inflammatory/immunologic cascades of events leading to the induction of allergic or irritant contact dermatitis or hyperkeratosis (1). A commonly used model in testing the animal's or human's ability to mount a cutaneous immune response is contact hypersensitivity (CHS) reaction to skin reactive chemicals, haptens. The afferent phase of the CHS response, the sensitization phase, is initiated by epicutaneous application of the hapten to the dorsal or abdominal skin, and it is characterized by the activation and division of hapten-specific T lymphocytes in the regional lymph nodes and the appearance of effector cells in the lymph nodes and spleen (2). In the efferent phase, which follows a subsequent challenge with hapten, e.g. epicutaneous application of the sensitizer to the skin of the ear, primed T lymphocytes are recruited to the site of challenge where they produce a variety of inflammatory mediators, amplifying a background inflammatory response into a more vigorous process. It is the classical manifestation of con-

tact hypersensitivity that is measured as "ear swelling". Ear swelling is the early-recognized skin response to hapten application, characterized histologically by dermal cell infiltration (3).

Epicutaneous application of haptens is commonly used test-system for antigen-specific T cell-mediated immune responses. Another type of T cell-mediated cutaneous hypersensitivity is delayed type hypersensitivity (DTH), widely used in testing cellular immunity to antigens following their subcutaneous or intradermal injection. Both CHS and DTH responses are often referred to as equivalent reactions, with the terms being used interchangeably and their distinctiveness reflecting only the types of antigen used and their route of administration (2). There is a growing body of evidence, however, that CHS and DTH are different reactions as there are data demonstrating that different cytokines (IL-10 vs. TNF-α) regulate CHS and DTH, respectively (4), and that differences exist regarding the complexity of effector cell participation pattern in these reactions (5). It was shown further that non-antigen specific, proinflammatory effects of haptens contribute to the elicitation of CHS (6) highlighting the importance of mechanisms of innate immunity in this reaction. In this regard, the involvement of granulocytes in CHS was stressed as depletion of these cells prior to elicitation inhibited the CHS expression (7). Moreover, it was shown that sensitization with hapten resulted in the enhancement of peripheral blood granulocyte activity (8). Taken together, these data further support the hypothesis that contact sensitivity is not a classic delayed type hypersensitivity and that it might be viewed as a cutaneous reaction in which both antigen (hapten)-specific and non-specific, innate immune mechanisms are engaged.

In the present study, the rat model of contact hypersensitivity to dinitrochlorobenzene (DNCB), developed previously in our laboratory (9), was employed to determine both aspects of the CHS response. As hapten sensitization occurs centrally, within draining lymph nodes (10) relevant parameters of draining lymph node cell (DLC) activity including spontaneous and hapten-stimulated proliferation and cell subset composition were determined following the sensitization. As an involvement of granulocytes in the elicitation was suggested (7) peripheral blood granulocyte activity following the elicitation of CHS was studied also. By using a rat model of CHS, limitations of the mouse CHS reaction regarding peripheral blood granulocyte accessibility were circumvented. Thus, basic functional aspects of peripheral blood granulocytes (activation, adhesion and cell survival) during the elicitation phase of CHS were determined.

Table 1. Changes in the draining lymph node cells during the induction of CHS reaction

	Treatment	
	Vehicle	DNCB
DLN cellularity (x10^6 cells)	13.32 1.88	70.88 13.88**
TCR (alpha/beta)[a]	84.42 ± 3.79 (57 ± 15.56)	79.72 ± 2.88 (68.82 ± 1.82)
CD4 [a]	55.12 ± 6.62 (58.64 ± 2.63)	44.74 + 1.55*** (77.19 ± 3.24***)
CD8 [a]	21.8 ± 4.76 (111.25 ± 9.22)	31.22 ± 1.01** (130.24 ± 5.78**)
CD4/CD8	1.97 ± 0.34	1.43 ± 0.02***
Dendritic cells in DLC population[b]	0.08 0.03	0.36 0.06***
Spontaneous proliferation rate of DLC (c.p.m. x 10^3)	437158	1734724059***
Hapten stimulated proliferation rate (Δc.p.m.)[c]	negligible	13158+752
Interleukin-2 stimulated proliferation rate (Δc.p.m.)[c]	1357 29	205625780***

aValues are given as mean percentage of immunofluorescence positive cells SD and as mean SD of mean fluorescence intensity (MFI) in parentheses; bValues are given as a mean percentage SD of dendritic cells per pool of draining lymph nodes; cc.p.m. = total c.p.m. minus c.p.m. in medium
*p<0.05; **p<0.025; ***p<0.01 versus vehicle

Materials and methods

The experiments were performed on inbred male Albino Oxford (AO) rats (Farm for Experimental Animals, Military Medical Academy, Belgrade, Yugoslavia) in adherence to the NIH guidelines for the use of experimental animals, with a permission of the Ethical Committee of our Institute. Groups of 6 to 8 rats received 100 μL of 2% w/v of 1-chloro-2,4-dinitrochlorobenzene, DNCB (BDH Chemicals LTD, England) dissolved in vehicle (acetone:olive oil 4:1) or an equal volume of vehicle solely, on the shaved dorsum for 2 consecutive days as described (9). Three days following sensitization, the rats were challenged by an application of 50 μL of suboptimal dose (0,66%) of DNCB to the outer half of the left ear. Ear thickness was measured with an engineer micrometer 24 hours after the challenge. The intensity of the response was quantified as the difference in the thickness between the challenged and non treated ears of the same animal and expressed as a percent of increase in ear thickness. Cartilage-free halves of exposed ears were fixed, stained with haematoxyllin and eosin for histological evaluation. Density of the dermal infiltrate was measured by the computer-assisted image analysis system using software "MIKRO" (Laboratory for the computer systems, Institute "Mihajlo PUPIN", Belgrade).

Draining (axillary and suprascapular) lymph node cell activity and phenotype were determined 72 h following sensitization. Suspensions of DLNC were prepared in the complete medium containing RPMI-1640 culture medium (ICN Flow, ICN Biomedicals Inc., Costa Mesa, CA, USA) supplemented with 5% heat-inactivated fetal calf serum (FCS) (ICN Flow), 1% gentamycin and $5x10^{-5}$ M 2-mercaptoethanol. DLNC were cultured for 24 hours in the medium only or in the presence of 50 IU/ml of recombinant human IL-2 (Genzyme, MA, USA) (for spontaneous proliferation and IL-2-stimulated proliferation, respectively) or 4 days in the presence of 5 μg/ml of dinitrobenzene sulfonic acid, DNBS (BDH Chem, UK), a water soluble analogue of DNCB (hapten-stimulated proliferation) at 37°C in a humidified atmosphere containing 5% CO_2. Proliferation was estimated by incorporation of ^{3}H thymidine (1 μCi/culture, Amersham, UK) added at the onset (spontaneous proliferation) or during the last 16-18 h of culture (IL-2-stimulated and DNBS-stimulated proliferation). Results are expressed as counts per minute (c.p.m.) measured by β-radioactivity scintillation counter (LKB RACK-Beta).

For the flow cytometry suspensions of DLNC in phosphate buffered saline were incubated with monoclonal antibodies R73 (anti-rat TCR α/β), W3/25 (anti-rat CD4), OX-8 (anti-rat CD8), all purchased from Serotec, Ltd, Bicester, UK, followed by rabbit anti Ig-FITC conjugated antibody (INEP, Zemun, Yu). Cells were analyzed for fluorescence intensity on an FACScan flow cytometer (Becton Dickinson, Germany). Results are expressed as percentages of fluorescence positive cells.

Peripheral blood granulocytes were isolated from the heparinized blood withdrawn from the abdominal aorta, 24 h following challenge, by dextrane sedimentation (6% w/v dextrane Pharmacia, Uppsala, Sweden), density gradient centrifugation (NycoPrep Animal 1007, Nycomed AS, Oslo, Norway) followed by lysis of erythrocytes with isotonic NH_4Cl solution. The remaining granulocytes were washed twice, resuspended in complete RPMI-1640 culture medium (without 2-mercaptoethanol) and held at room temperature for functional studies. The purity of the neutrophils was more than 95%, as determined by May-Grunwald-Giemsa staining.

A quantitative colometric assay described for human granulocytes (11) in which tetrazolium salt MTT is metabolically reduced to colored end product, formazan, was employed to estimate cell survival of rat peripheral blood granulocytes. MTT reduction was estimated immediately (in freshly isolated cells) or following 18 h in culture. Formazan produced was dissolved in SDS-Cl and absorbency was measured at dual wavelength, 570nm/650nm by an ELISA 96-well plate reader (Behring ELISA Processor, Behring, FRG). Cell survival was expressed as MTT index (absorbance of formazan solubilized at the end of incubation period divided by absorbance at the onset of culture).

Spontaneous and phorbol 12-myristate 13-acetate (PMA, 50 ng/ml) (Sigma Chemical Co., St. Louis, Mo., USA) stimulated activation of granulocytes was evaluated by a cytochemical assay for the respiratory burst (12), which is the measure of an intracellular reduction of nitroblue tetrazolium (NBT). Formazan produced by granulocyte reduction of NBT was extracted with acidified SDS and absorbance measured spectrophotometrically as described above. Adhesion of granulocytes to plastic was assessed by using a modified assay initially described by Oez (13) in which the 60-minute adhesion of granulocytes to plastic of a 96-microwell plate was measured. After incubation in the medium (spontaneous adhesion) or in the presence of 50 ng/mL PMA and washing off non-adherent cells, adherent cells were stained with 0.1% methylene blue. The absorbance of acid-dissolved dye was measured at 650 nm/570 nm by an ELISA 96-well plate reader.

Data are expressed as mean value ±SD for each experimental animal group (5-7 animals) for the determination of ear swelling and DLNC phenotype. In the DLNC and granulocyte functional assays, data represent means ±SE for triplicates or quadruplicates from single experiments representative out of three. Significance was defined by Student's t-test. p values less than 0.05 were considered significant.

Results

Afferent phase of the contact hypersensitivity reaction: draining lymph node cell activity

Changes in draining lymph node cells 72 hours following local epicutaneous application of 2% DNCB were determined as preliminary experiments had demonstrated a maximal spontaneous proliferation rate of DLNC at this time point. The data are summarized in Table 1. A shift in the ratio of $CD4^+$ to $CD8^+$ DLNC was noted on day 3 following the DNCB application, due to a decrease in relative numbers of $CD4^+$ T cells and an increase in relative numbers of $CD8^+$. Mean fluorescence intensity of cell surface CD4 and CD8 antigens on DLNC was increased in the DNCB-treated group compared to the vehicle-treated group. As increased cellularity of draining lymph nodes of sensitized animals was noted, absolute numbers of cells from these subsets greately outnumbered those in draining lymph nodes of non-sensitized animals. An enrichment in cells with distinct dendritic morphology was observed in DLNC from sensitized animals. Significantly increased levels of spontaneous proliferation as well as IL-2-stimulated and DNBS-stimulated proliferation were detected in cultures of DLNC from sensitized animals, which could be ascribed to increased percentages of $IL\text{-}2R^+$ cells in the sensitized compared to the vehicle treated animals (8.6±0.99 vs. 5.50±1.27).

Efferent phase of contact hypersensitivity reaction

Ear swelling

Ear swelling assay as well as descriptive and quantitative histology were determined 24 hours following challenge with DNCB. Nearly a 10-fold increase (108.2±39.3% vs. 9.51±5.96%, $p<0.05$) in ear swelling was noted following the application of 0.66% DNCB. This increase was accompanied by an inflammatory response in the exposed skin characterized by epidermal changes, similar to the well-known picture of allergic contact dermatitis, including vacuolisation of the basal layer, and spongiosis with formation of microvesicles at the dermo-epidermal junction. Subtle superficial crusts were also seen. Density of the dermal infiltrate, measured by the computer-assisted image analysis system, was increased compared to the control, vehicle treated group (22.81±0.81 cells/area vs. 13.67±3.4 cells/area, $p<0.05$) in which all of the described epidermal and dermal changes were not seen.

Peripheral blood granulocyte activity in the elicitation phase of CHS

Changes in peripheral blood (PB) granulocyte function were demonstrated following a challenge with suboptimal doses of DNCB and are presented in Table 2. Increased MTT index was noted in cultures of granulocytes from sensitized animals. Both spontaneous and PMA-stimulated NBT reduction were increased following challenge with 0.66% DNCB. A stronger PMA-stimulated adhesion of granulocytes was noted in DNCB-treated group.

Discussion

In this study, parameters of both hapten-specific as well as innate immune responses were determined in experimentally induced contact sensitivity in rats. Enrichment in the dendritic cell contents in lymph nodes that drain the area of epicutaneous DNCB application is in concordance with data from numerous studies conducted in mouse models of CHS which demonstrated the appearance of dendritic cells in regional lymph nodes following hapten application (2). An increase in cellularity of draining lymph nodes, a commonly used parameter of local lymph node activity (14, 15) might be a result of either lymphocyte proliferation or local recruitment of antigen-specific lymphocytes or both. Lymphocyte proliferation is evident *in vitro* as increased spontaneous proliferation and ocurring in response to exogenous IL-2 and DNBS.

Phenotypic characterization of DLNC showed increased numbers of draining lymph node cells expressing CD4 and CD8 antigens. This is in line with investigations linking the effector stages of CHS with $CD8^+$ (16-19) and/or $CD4^+$ T cells (5, 20). The upregulation of cell surface expression of CD4 may be in accordance

Table 2. Changes in the granulocyte activity during elicitation of CHS reaction

	Treatment	
	Vehicle	DNCB
MTT index	0,73±0,02	0,93±0,00**
NBT spontaneous	0.054±0.0	040.066±0.001*
PMA stimulated	0.199±0.001	0.235±0.002*
Adhesion spontaneous	0.015±0.002	0.019±0.003
PMA stimulated	0.026±0.001	0.034±0.003*

Values are given as mean ±SE
*$p<0.05$ and ** $p<0.01$ versus vehicle

with a study which found a correlation between CD4 density and the functional activity of $CD4^+$ in the CHS response (5). Similarly, the observed increase in cell-surface density of CD8 might also be related to the involvement of the cells in the effector arm of CHS.

On the basis of parameters of draining lymph node activity, as well as on the elicitation phase of reaction, the rat model of CHS reaction presented is compatible with the mouse model and could be used, as recommended earlier, as a suitable model for cell-mediated immunity studies in experimental contact allergy (21).

Recent studies have brought evidence on the involvement of innate immunity/inflammatory responses in CHS. It was shown that antigen non-specific, proinflammatory effects of hapten contribute to the elicitation of CHS (6) and that the dual (irritant and antigenic) properties of sensitizing chemicals are needed for the development of allergic contact dermatitis (22). In this regard, the importance of granulocytes in the effector phase of CHS was suggested (7).

The rat model of CHS enabled analysis of peripheral blood granulocyte function in the elicitation of CHS. The functional state of peripheral blood granulocytes in the elicitation phase of CHS was examined by *in vitro* tests of granulocyte activation, adhesion and survival. The potential of granulocytes to reduce tetrazolium salts to formazan *via* tetrazolium reducing respiratory burst oxidase (23) was used as an *in vitro* measure of granulocyte activation (12). It showed increased spontaneous activation of granulocytes from challenged animals. Significantly higher responses provoked with suboptimal PMA (50 ng/ml) in granulocytes from challenged animals compared to those seen in controls, suggest a primed state of the granulocytes (24).

Adhesiveness of granulocytes to various matrices is commonly used test of granulocyte activity, as an *in vitro* correlate of granulocyte adherence to endothelium or connective tissue matrices (13). Plastic microtitre plates are widely used in the assessment of granulocyte adhesion as plastic surfaces behave physiologically with respect to adhesiveness of granulocytes induced by various stimuli (13, 25). There was no difference in spontaneous adhesion of granulocytes from challenged compared to vehicle-treated animals. However, significantly increased adhesion of granulocytes from challenged *vs* control animals following stimulation with PMA, a known stimulus of granulocyte adhesion (26), may reflect a higher propensity of granulocytes from challenged rats to adhere.

The increase in activation of peripheral blood granulocytes following challenge with DNCB might have resulted from the cutaneous toxicity of DNCB and higher demands for granulocytes at the site of hapten application. Dermatotoxicity of DNCB is well known (27, 28). Increased activity in reduction of NBT following elicitation of CHS, might also resulted from previous activation of peripheral blood granulocytes as demonstrated to occur following sensitization with DNCB (8).

Our data demonstrated that the increased functional status of peripheral blood granulocytes following challenge with DNCB was accompanied by the increased survival of these cells. As terminally differentiated cells, peripheral blood granulocytes die rapidly in culture (29), but their survival might be modulated in conditions of inflammation (30, 31), where these cells are exposed to various inflammatory mediators, including cytokines (32, 33). Skin sensitization with haptens was demonstrated to increase levels of circulating IL-6 (34) a cytokine, which might delay apoptosis of PMNs (30). Thus, the increased index of spontaneous MTT reduction in cultures of granulocytes from challenged rats, might result from a previous exposure of granulocytes to inflammatory mediators, including cytokines, in the circulation. Our data, which demonstrated a prolonged peripheral blood granulocyte survival following sensitization of rats with DNCB, further increased following *in vitro* granulocyte incubation with autologous plasma, support this assumption (8).

Overall, these findings support a role of granulocytes in the elicitation of CHS. Indeed, depletion of granulocytes before the elicitation prevented the expression of CHS in mice (7). As changes in peripheral blood granulocyte activity were noted during the sensitization phase also (8), these, along with the presented data strengthen a contributory role of these cells in contact sensitivity. In this regard, a role for skin-infiltrating neutrophils in the recruitment of hapten-specific $CD8^+$ T cells to the site of hapten challenge was suggested (7).

Taken together, these data imply that experimental contact hypersensitivity might be viewed not only as a system for studying the role of hapten-specific but for non-specific mechanisms of this cutaneous response, as well.

One further aspect of the rat CHS model should be mentioned. It is a frequently used experimental animal model in toxicity and immunotoxicity studies (14, 35, 37, 38) and in testing of chemicals and drugs to be approved for use in humans (35, 36). In this regard, studying hapten-specific as well as non-specific aspects of this cutaneous reaction following a xenobiotic exposure might give better insights into mechanisms of immune modulation by these agents.

In summary, the contact hypersensitivity reaction induced by DNCB in rats represents, in our opinion, a suitable experimental model for studying both antigen-specific and antigen-nonspecific mechanisms of cutaneous immune responses to contact sensitizing chemicals. It may prove to be very useful for studying both local and systemic immunomodulatory/immunotoxic effects of various chemicals and physical agents *in vivo*. Examination of these effects in the same animal model might represent a more integrated approach to the immunodermatotoxicological research.

REFERENCES

1. Rheins A. What's new in cutaneous toxicity. J Toxicol Cut Ocular Toxicol 1992; 11: 225-38.

2. Lappin MB, Kimber I, Norval M. The role of dendritic cells in cutaneous immunity. Arch Dermatol Res 1996; 288: 109-21.

3. Roupe G, Ridell B. The cellular infiltrate in contact hypersensitivity to picryl chloride in the mouse. Acta Dermatol Venereol (Stockholm) 1979; 59: 191-5.

4. Kawabata TT, Burleson GR, Ernst PB, Ullrich SE. Immunotoxicology of regional lymphoid tissue: The respiratory and gastrointestinal tracts and skin. Fund Appl Toxicol 1995; 26: 8-19.

5. Gocinski BL, Tigelaar RE. Roles of $CD4^+$ and $CD8^+$ T cells in murine contact sensitivity revealed by in vivo monoclonal antibody depletion. 1990; 144: 4121-8.

6. Grabbe S, Steinert M, Mahnke K, Schwartz A, Luger TA, Schwarz T. Dissection of antigenic and irritative effects of epicutaneously applied haptens in mice. Evidence that not the antigenic component but non-specific proinflammatory effects of haptens determine the concentration-dependent elicitation of allergic contact dermatitis. J Clin Invest 1996; 98: 1158-64.

7. Dilulio NA, Engeman T, Armstrong D, Tannenbaum C, Hamilton TA, Fairchild RL. Groalpha-mediated recruitment of neutrophils is required for elicitation of contact hypersensitivity. Eur J Immunol 1999; 29: 3485-95.

8. Kataranovski M, Drašković-Pavlović B, Jovičić G, Milojević G, Todorović V, Colić M, Popović P. Peripheral blood granulocyte aactivity following contact sensitization of rats with dinitrochlorobenzene. Toxicology 2001 (in press).

9. Kandolf L, Kataranovski M, Berger S, Milosavljević I, Karadaglić Đ. Experimentally induced contact hypersensitivity reaction to dinitrochlorobenzene (DNCB) in rats. Arch Toxicol Kinet Xenobiot Metab 1998; 6: 287-8.

10. Kripke ML, Munn Cg, Jeevan A, Tang J-M, Bucana C. Evidence that cutaneous antigen-presenting cells migrate to reghional lymph nodes during contact sensitization. J Immunol 1990; 145: 2833-8.

11. Oez S, Welte K, Platzer E. A quantitative colorimetric method to evaluate the functional state of human polymorphonuclear leukocytes. Blut 1990; 60: 97-102.

12. Monboisse J-C, Garnotel R, Randoux A, Dufer J, Borel J-P. Adhesion of human neutrophils to and activation by type-I collagen involving a 2 integrin. J Leukoc Biol 1991; 50: 373-80.

13. Oez S, Welte K, Platzer E Kalden JR. A simple assay for quantifiying the inducible adherence of neutrophils. Immunobiol 1990; 180: 308-15.

14. Arts JH, Droge SC, Bloksma N, Kuper CF. Local lymph node activation in rats after dermal application of the sensitizers 2,4-dinitrochlorobenzene and trimellitic anhydride. Food Chem Toxicol 1996; 34: 55-62.

15. Kimber I, Weisenberger C. A murine local lymph node assay for the identification of contact allergens. Assay development and results of initial validation study. Aarch Toxicol 1989; 63: 274-82.

16. Xu H, Dilulio NA, Fairchild RL. T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon-γ-producing (Tc1) effector $CD8^+$ T cells and interleukin (IL) 4/IL-10 producing (Th2) negative regulatory CD4+ T cells. J Exp Med 1996; 183: 1001-12.

17. Lopez CB, Kalergis AM, Becker MI, Garbarino JA. $CD8^+$ T cells are the effectors of the contact dermatitis induced by urushiol in mice and are regulated by $CD4^+$ T cells. Int Arch Allergy Immunol 1998; 117: 194-201.

18. Cavani A, Mei D, Guerra E, Corinti S, Giani M, Pirrotta L, Puddu P, Girolomoni G. Patients with allergic contact dermatitis to nickel and nonallergic individuals display different nickel-specific T cell responses. Evidence for the presence of effector $CD8^+$ and regulatory $CD4^+$ T cells. J Invest Dermatol 1998; 111: 621-8.

19. Martin S, Lappin MB, Kohler J, Delattre V, Leicht C, Preckel T, Simon JC, Weltzien HU. Peptide immunization indicates that CD8+ T cells are the dominant effector cells in trinitrophenyl-specific contact hypersensitivity. J Invest Dermatol 2000; 115: 260-6.

20. Bunce C, Bell EB. CD45 isoforms define two types of CD4 memory T cells, one which depends on persisting antigen. J Exp Med 1997; 185: 767-76.

21. Scheynius A, Skoglund C, Johanson C, Holmdahl R. The rat as an experimental model for studies on immune regulating mechanisms in contact allergic reactions. Acta Derm Venereol (Suppl.) 1988; 135: 54-56.

22. Zhang L, Tinkle SS. Chemical activation of innate and specific immunity in contact dermatitis. J Invest Dermatol 2000; 115:168-76.

23. Kakinuma K, Fukuhara V, Kaneda M. The respiratory burst oxidase of neutrophils. J Biol Chem 1987; 262: 12316-24.

24. Hallet M, Lloyds D. Neutrophil priming: the cellular signals that say "amber" but not "green". Immunol Today 1995; 16: 264-8.

25. Forsyth K, Levinsky RJ. Role of the LFA-1 adhesion glycoprotein in neutrophil adhesion to endothelium and plastic surfaces. Clin Exp Immunol 1989; 75: 265-8.

26. Arnaout A. Structure and function of the leukocyte adhesion molecules CD11/CD18. Blood 1990; 75: 1037-50.

27. Nakamura M, Rikimaru T, Yano T, Moore G, Pula PL, Shofield BH, Dannenberg AM. Full-thickness human skin explants for testing the toxicity of topically applied chemicals. J Invest Dermatol 1990; 95: 325-32.

28. Luster MI, Wilmer JI, Germolec DR, Spalding J, Yoshida T, Gaido K, Simeonova PP, Burleson FG, Bruccoleri A. Role of keratinocyte-derived cytokines in chemical toxicity. Toxicol Lett 1995; 82-83: 471-6.

29. Haslett C. Resolution of acute inflammation and the role of apoptosis in the tissue fate of granulocytes. Clin Sci 1992; 83: 639-48.

30. Biffl WL, Moore EE, Moore FA, Barnett CC Jr, Carl VS, Peterson VN Interleukin-6 delays neutrophil apoptosis. Arch Surg 1996; 40: 217-25.

31. Kataranovski M, Popović S. Differential effects of in vivo cadmium administration on lymphocytes and granulocytes in rats. Vet Human Toxicol 1999; 41: 200-4.

32. Collota E, Re F, Polentarotti N, Sozzani S, Mantovani A. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. Blood 1992; 15: 2012-20.

33. Chintis D, Dickerson C, Munster AM, Winchurch RA. Inhibition of apoptosis in polymorphonuclear neutrophils from burn patients. J Leukoc Biol 1996; 59: 835-9.

34. Kimber I, Cumberbatch M, Humpreys M, Hopkins SJ. Contact hypersensitivity induces plasma Interleukin 6. Int Arch Allergy Appl Immunol 1990; 92: 97-9.

35. Koller LD, Exon JH. The rat as a model for immunotoxicology assessment. In: Dean J et al., editors. Immunotoxicology and Immunopharmacology. New York: Raven Press 1985: 99-111.

36. Gill TJ III, Smith, GJ, Wissler RW, Kunz HW. The rat as an experimental animal. Science 1989; 245: 269-76.

37. Lauerma AI, Homey B, Vohr HW, Lee CH, Bloom E, Maibach HI. Effect of topical cis-urocanic acid on local lymph node activation during contact sensitization in mouse, rat and guinea-pig. Br J Dermatol 1996; 134: 904-9.

38. Nelson CJ, How T, Lysle DT. Enhancement of the contact hypersensitivity reaction by acute morphine administration at the elicitation phase. Clin Immunol 1999; 93: 176-83.

AUTHORS' ADDRESSES

Milena Kataranovski PhD, Professor of immunobiology, Faculty of Biology, University of Belgrade, Studentska 4, and Senior Research associate, Institute for Medical Research, Military Medical Academy, 17 Crnotravska, 11002 Belgrade, Yugoslavia, e-mail: vmaimi@eunet.yu
Lidija Kandolf Sekulović MD, Department of Dermatology, Military Medical Academy, 17 Crnotravska, 11002 Belgrade, Yugoslavia, e-mail: bdd_sld@yubc.net
Đorđije Karadaglić MD, PhD, Professor of Dermatology, Department of Dermatology, Military Medical Academy, 17 Crnotravska, 11002 Belgrade, Yugoslavia, e-mail: bdd_sld@yubc.net