STATE OF IMMUNOREACTIVE SYSTEMS DURING HEALING OF ASEPTIC AND INFECTED EXPERIMENTAL WOUNDS

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Despite continuous interest in the problem of wounds and wound infections, and the abundance of publications devoted to its study, determination of the character of the course of wound healing and therapeutic tactics have not been finally settled, and they continue to be topics for unceasing discussion [4, 10]. The functional state of the immunoreactive systems, both of the body as a whole and in the zone of wounding, exerts a considerable influence on the course of wound healing [7, 11]. Besides the study of the response of other systems, it is therefore essential to take account of genetically determined forms of immune response and of nonspecific reactive systems to stressors, which include both experimental and clinical wounds .The combined study of these mechanisms of response enabled approaches to the correction of cellular and humoral components determining the course of wound healing to be objectivized and pathogenetically based not only as regards the treatment of wounds, but also the prevention of their complications. Although this is such a promising approach, considerable difficulties arise in the path of its realization and, in our view, connected with the absence of any clear criteria of the dynamics of the parameters of immunoreactivity during development and healing of wounds at the present time, for the phenomenology of these principles has so far been described for suppurative wounds with many concomitant factors (diabetes, diversity of the microflora, heterogeneity of stages of the process, difference of localization, and so on). Meanwhile these changes also take place during "aseptic" healing of postoperative wounds and of everyday microtraumas. Consequently, the comparative study of immune changes in aseptic and suppurative wounds may shed light, on the basis of differences discovered, on those critical factors to which correction ought to be directed. The aim of the present investigation was to study these matters.

EXPERIMENTAL METHOD

Two series of experimenta were carried out on 240 male Wistar rats weighing 200-210 g. Aseptic and infected full-thickness wounds with an area of 400 mm^2, produced with the aid of Teflon rings by the method described previously [9], served as the exerimental model. At intervals before the operation (background) and on the 1st-10th, 12th, and 15th days after it, the following parameters of immunological activity were studied in the blood: T and B lymphocytes in rosette formation tests [13, 14]; the "active" subpopulation of T lymphocytes forming rosettes at 37°C [15]. Hemolytic activity of complement was assessed on the basis of 50% hemolysis [6]. The serum lysozyme level was determined by a turbidimetric method [5], β-lysins by a photonephelometric method [1], and bactericidal activity of the serum (BAS) also by a photonephelometric method [8]. Phagocytic activity of the neutrophils was studied by a direct visual method with simultaneous determination of the bactericidal capacity of the neutrophils [3]. A living 24-h culture of *Staphylococcus*, strain No. 9198, was used as the object of phagocytosis; opsonization was not used. For quantitative characterization of phagocytosis the phagocytic index, i.e., the percentage of phagocytosing neutrophils (PI_{30} and PI_{120}), was determined 30 and 120 min after the beginning of incubation with the microorganisms; the phagocytic number, i.e., the number of microorganisms per phagocyte (PN_{30} and PN_{120}), also was determined. The culmination of phagocytosis and digestion of the microorganisms were judged by the coefficient of the phagocytic number (CPN), i.e., the ratio of PN_{30} to PN_{120} (phagocytosis was considered to be complete when CPN > 1). Bactericidal capacity of

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TABLE 1. Changes in Some Immunological Parameters of Blood during Healing of Aseptic Wounds in Rats

Parameters \ Times	0 (background)	1	2	3	4	5	6	7	8	9	10	12	15
Phagocytic index 30 min	50,7±2,28	53,6±1,21	52,2±2,54	52,6±3,5	64,9±2,4	62,6±2,34	51,6±1,9	51,4±2,64	56,4±1,85	58,0±1,97	50,4±3,5	53,0±2,7	53,0±2,2
120 min	53,3±1,94	70,7±1,32	65,9±3,62	62,4±2,6	73,6±1,6	73,0±3,12	70,5±2,6	68,1±2,04	61,4±1,91	68,4±1,97	52,8±1,4	51,0±3,2	60,6±1,7
Phagocytic number, % 30 min	4,36±0,32	5,82±0,33	6,04±0,13	6,96±0,4	8,32±0,4	8,72±0,5	6,10±0,2	5,81±0,21	5,70±0,3	6,07±0,15	6,16±0,3	5,72±0,2	4,97±0,1
120 min	3,86±0,17	6,27±0,22	5,70±0,13	8,26±0,9	9,10±0,6	7,67±0,47	5,69±0,2	5,56±0,19	5,92±0,28	6,11±0,2	6,01±0,5	5,40±0,3	4,80±0,52
CPN	1,13±0,04	0,92±0,07	1,06±0,05	0,84±0,1	0,91±0,1	1,13±0,13	1,1±0,08	1,04±0,07	0,96±0,1	1,0±0,06	1,02±0,1	1,06±0,1	1,03±0,1
IBN, %	67,2±0,9	63,7±2,7	73,9±1,75	75,0±1,0	77,1±1,8	72,8±2,32	72,7±1,3	73,1±1,5	73,1±0,93	74,0±1,5	75,4±0,9	72,2±1,6	70,2±1,0
Hemolytic activity of complement (CH_{50})	39,9±0,94	38,9±1,15	40,2±1,73	40,4±1,1	39,4±0,74	37,8±0,35	39,5±0,51	38,8±0,70	39,1±0,51	37,9±0,70	38,9±1,94	37,5±1,3	39,9±0,44
Lysozyme, μg	2,36±0,34	6,01±0,6	7,6±0,35	8,3±0,24	6,62±0,97	3,3±0,08	4,83±0,9	0,90±0,2	2,12±0,3	2,53±0,4	2,21±0,5	2,24±0,4	2,8±0,35
Bacteriostatic effect of lysins, %	76,63±2,6	71,0±3,91	71,0±3,52	70,4±3,3	70,4±3,5	76,0±3,31	73,2±4,62	54,8±2,71	72,9±3,45	75,6±5,1	67,4±5,1	64,0±3,2	72,2±8,44
Lytic effect of lysins, %	62,37±2,7	63,7±2,44	66,0±3,0	70,6±2,3	75,4±1,7	73,0±2,6	66,1±2,04	56,4±2,9	60,2±4,7	67,9±2,98	67,3±3,4	67,6±2,5	67,3±1,7
BAS, %	66,4±2,43	73,2±1,93	73,6±2,44	73,1±2,1	73,5±3,0	74,0±3,6	75,6±2,55	66,3±4,3	66,0±4,2	65,5±2,12	62,0±4,5	61,2±1,5	61,8±3,6
T lymphocytes, %	17,0±0,8	8,4±0,7	3,6±0,6	5,42±0,9	9,0±0,92	10,3±0,6	12,9±1,3	11,6±0,8	9,62±0,32	7,12±0,71	6,14±0,6	8,14±1,0	9,62±0,32
B cells, %	60,9±2,3	46,6±2,6	30,0±2,5	20,7±2,1	40,5±2,0	56,1±2,5	73,1±3,85	41,4±2,83	41,0±1,33	39,0±1,09	38,7±1,7	36,7±2,2	29,1±1,9
T-active cells, %	8,0±0,6	5,85±0,73	4,14±0,5	4,6±0,9	5,75±0,8	6,6±0,5	7,5±0,65	6,75±0,53	6,62±0,5	6,25±0,92	4.85±0,4	3,6±0,6	5,43±0,52

TABLE 2. Changes in Some Immunological Parameters of the Blood during Healing of Infected Wounds in Rats

Parameters \ Times	0 (background)	1	2	3	4	5	6	7	8	9	10	12	15
Phagocytic index 30 min	50,7±2,28	49,2±1,8	53,1±2,2	53,6±1,2	60,7±1,4	60,1±1,06	56,7±1,7	52,0±2,3	52,0±1,81	80,0±2,29	46,7±0,6	46,4±0,5	46,5±1,3
120 min	53,3±1,94	61,2±1,7	67,3±0,96	65,5±1,3	68,2±1,3	67,6±1,6	55,7±1,6	54,8±1,72	72,2±1,8	77,6±1,42	66,8±1,1	63,6±1,8	49,9±1,8
Phagocytic number, % 30 min	4,37±0,32	7,5±0,23	6,3±0,20	6,14±0,15	6,0±0,2	6,09±1,07	6,0±0,12	5,53±0,16	6,9±0,2	6,7±0,3	5,5±0,09	5,45±0,11	5,44±0,1
120 min	3,86±0,17	6,59±0,3	5,45±0,18	5,6±0,15	5,7±0,16	5,3±0,2	4,2±0,22	4,4±0,11	6,84±0,16	7,76±0,34	5,43±0,2	5,45±0,09	5,04±0,13
CPN	1,13±0,04	1,13±0,1	1,15±0,1	1,1±0,06	1,1±0,06	1,15±0,2	1,2±0,08	1,26±0,07	1,01±0,05	0,86±0,08	1,0±0,04	1,0±0,04	1,04±0,04
IBN, %	67,2±1,4	76,6±1,3	70,7±2,01	65,6±2,04	77,0±1,32	78,0±1,2	76,6±1,4	70,2±1,15	66,0±0,05	67,8±0,2	67,4±1,9	68,2±0,2	67,5±1,5
Hemolytic activity of complement (CH_{50})	39,9±0,94	41,9±0,4	40,6±0,7	39,3±0,4	41,6±0,6	42,7±0,7	39,2±0,7	38,7±1,3	32,4±0,51	37,9±1,3	42,5±0,5	37,4±1,1	37,6±0,95
Lysozyme, μg	2,36±0,34	2,43±0,43	2,64±0,33	2,51±0,2	1,2±0,12	2,6±0,4	2,5±0,61	2,31±0,63	1,5±0,25	1,35±0,41	1,9±0,3	1,71±0,3	1,06±0,3
Bacteriostatic effect of lysine, %													
Lytic effect of lysins, %	76,63±2,6	79,2±1,97	73,4±3,52	73,5±2,94	72,0±3,17	74,1±4,6	76,6±2,8	65,4±3,43	52,6±2,16	61,3±2,6	62,3±5,2	64,1±3,2	75,7±3,6
BAS, %	62,4±2,65	67,9±2,9	68,2±2,6	64,7±2,9	60,7±3,13	60,9±1,4	64,7±2,8	57,7±3,9	52,7±2,44	47,3±4,4	52,0±3,9	54,4±3,12	64,3±4,3
T lymphocytes, %	63,4±2,43	77,9±2,9	76,7±1,71	73,2±2,5	70,6±2,1	65,9±4,2	65,6±3,3	70,7±2,0	65,4±4,3	52,4±1,71	60,6±3,2	61,4±1,73	67,0±2,6
	17,0±1,05	7,1±0,75	7,4±0,5	11,2±1,53	24,0±2,2	25,2±0,2	22,5±1,6	9,12±0,6	8,75±0,52	8,71±0,6	8,25±0,8	5,4±0,7	4,14±0,3
B cells, %	60,9±2,41	60,0±1,6	50,5±1,7	40,1±2,7	38,2±1,1	25,0±0,9	15,5±0,9	16,0±1,15	28,5±1,8	37,3±2,6	43,0±1,93	24,3±1,43	24,7±1,3
T-active cells, %	8,0±0,68	4,75±0,5	5,4±0,6	4,9±0,7	4,4±0,3	4,75±0,7	5,9±0,91	7,12±0,64	7,25±0,8	6,62±0,6	6,12±0,56	5,4±0,62	5,12±0,8

the neutrophils was judged by the index of bactericidal activity of the neutrophils (IBN), the ratio of the number of killed microorganisms lying intracellularly to the total number of microorganisms phagocytosed by neutrophils, expressed in per cent. Blood for investigation and for preparation of serum was taken from femoral vessels. The rats were put to sleep with hexobarbital (1%, 3.0 mg, intramuscularly). At each time point 8-10 animals were used, and after blood sampling they were killed by decapitation. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Factors of Natural Resistance. Comparative analysis of parameters of phagocytic activity and serum factors of nonspecific reactivity revealed marked differences between the course of aseptic and infected wounds. The time course of the phagocytic parameters (Table 1) shows clear dependence of the number of neutrophils taking part in phagocytosis, i.e., the phagocytic index, and the level of digestive activity of the neutrophils (FN_{30} and FN_{120}) on temporal parameters of the nonspecific pressor reaction observed during healing of aseptic wounds. The influence of the pressor effect on the increase in lytic effects of β-lysins was most demonstrative, and coupled with the absence of changes in their bacteriostatic effect with time, this determines the positive biological trend of the phenomenon thus revealed. The fact that the increase in the bacteriological activity of the β-lysins at consecutive times coincided with activation of proliferative processes (3rd-5th days) in aseptic wounds [4] is evidence that the trend of action of β-lysins is directly connected with the level of functional (secretory) activity of the macrophages. This was confirmed by a distinct rise of the lysozyme level on the 1st-6th days, with a peak on the 3rd day.

A different picture was observed during healing of infected wounds: the parameters of phagocytic activity showed a similar trend to that described above (Table 2), but it was much weaker, and in the later stages (after 10 days) they showed a significant fall. In must be particularly emphasized that the bactericidal activity of the neutrophils and their digestive capacity (reflected in the value of CPN), and, a matter of particular importance from our point of view, the lytic effect of β-lysins and BAS, as an integral parameter reflecting total antibacterial activity of serum factors, were virtually unchanged (at least within significant intervals).

It will be clear from Table 1 that the level of hemolytic activity (CH_{50}) of complement remained virtually unchanged and did not differ significantly from the background value throughout almost the whole period of observation during healing of aseptic wounds. On the 4th-5th day of healing of infected wounds, complement titers were raised ($p < 0.05$). The difference between the series at this period likewise was significant. On the following days the complement titers fell and reached a minimum on the 8th day. A further rise was observed on the 10th day. This mosaic pattern of changes of complement titers may be evidence of the phasic nature of changes in the parameters of natural resistance of the body under the influence of metabolic products of microorganisms in the wound area. The suggestion likewise cannot be ruled out that the level of the parameter of hemolytic activity of complement reflects the trend of development of the microbial flora in the wound.

The Action of Lymphocytes during Healing of Aseptic and Infected Wounds. The relative parameters (percentages) of B lymphocytes were significantly depressed almost throughout the period of observation in both series (Tables 1 and 2). However, the course of the cellular response during healing of an aseptic wound shows an increase in the relative percentage of B cells on the 5th-7th day, whereas the opposite picture was observed with equal temporal parameters during healing of infected wounds. A different picture was observed in the study of the kinetics of the T-cell response. During healing of aseptic wounds there was a marked decrease in the relative number of T cells, especially on the 2nd-3rd days. Later a definite tendency was observed for this population to increase, although at the end of the experiment it has not yet returned to the background value. By contrast with aseptic wounds, during healing of infected wounds the number of T cells increased considerably in the course of 3-6 days. The quantitative changes in immunocompetent cells described above were manifested most clearly when they were compared with the morphological data, and also with parameters of nonspecific reactivity. In the period of most intensive granulation (5th-7th days), i.e., in the period of activation of the macrophagal-monocytic system, nonspecific stimulation of B cells takes place. It is short in duration and coincides with periods of activation of macrophages during this period of wound healing. During healing of infected wounds, however, bacterial toxins and other products of metabolism and degradation of microorganisms cause the levels of these cells to fall during the

same time intervals. Yet we know that bacterial antigens are T-dependent [12]. It is this fact which can explain activation of the T-cell clone wjich is observed during the period of intensive development of suppurative inflammation in infected wounds. The small number of T cells in rats with aseptic wounds reflects the well known reduction in the number of T cells in the peripheral blood during the first days of exposure to stress [2].

It may also be suggested that not only may the local effect of the bacterial flora change the course of repair processes, by acting along with other factors on immunocompetent cells, but the state of the immune system of the body may also predetermine the development of suppurative inflammation. The particular feature of the time course of the immune response discovered by the writers previously [11], reflecting functional insufficiency of the immunoreactive systems, led to the suggestion of a method of assessing the immunological characteristics of wound healing in order to predict suppurative complications in the postoperative period in surgical practice.

The results obtained experimentally thus show conclusively that an aseptic wound induces a nonspecific pressor response of the body, but the presence of a bacterial flora in it inhibits the development of the humoral immune response against the background of T-cell activation, and thus causes dysfunction of the immune system, which is reflected in the course of wound healing. It can be tentatively suggested that the degree of this dysfunction depends on the character of the microbial flora and also on the functional state of the immune system. If these factors are taken into consideration in surgical practice a step will have been taken toward the solution of the urgent problem of reducing the incidence of suppurative complications in the postoperative period.

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