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Vitamin A Deficiency Predisposes to *Staphylococcus aureus* Infection

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We have investigated the consequences of vitamin A deficiency in a rat model of T-cell-dependent and superantigen-mediated *Staphylococcus aureus* arthritis. After intravenous inoculation of enterotoxin A-producing staphylococci, the vitamin A-deficient rats showed a decreased weight gain compared with the paired fed controls despite equal food consumption. The control rats developed arthritis in the first few days after bacterial inoculation, with a peak frequency at day 5, and then gradually recovered; however, the frequency of arthritis in the deficient rats increased continuously during the experimental period. The prevalence of arthritis 18 days after bacterial inoculation was 86% among the vitamin A-deficient rats and 44% among the control rats. During this period, 3 of 10 deficient rats and 1 of 10 control rats died. Further in vitro analysis revealed that T-cell responses to *S. aureus* were significantly higher in the vitamin A-deficient rats than in the control animals. In contrast, B-cell reactivity, measured as immunoglobulin levels, autoantibody levels, and specific antibacterial antibody levels in serum, did not differ between the groups. Interestingly, the innate host defense mechanisms against *S. aureus* were also profoundly affected by vitamin A deficiency. Thus, despite a larger number of circulating phagocytic cells in the vitamin A-deficient group, the capacity to phagocytize and exert intracellular killing of *S. aureus* was significantly decreased in comparison with the control rats. Furthermore, serum from the vitamin A-deficient rats inoculated with *Staphylococcus aureus* displayed decreased complement lysis activity. Our results suggest that the increased susceptibility to *S. aureus* infection observed in the vitamin A-deficient rats is due to a concerted action of antigen-specific T-cell hyperactivity, impaired function of the phagocytes, and decreased complement activity.

Clinical studies concerning the risk of acquiring bacterial infections during vitamin A deficiency show conflicting results. Whereas West et al. (32) and Sommer et al. (30) have shown that subjects with vitamin A deficiency display an increased incidence and severity of infections, other studies have challenged these conclusions by claiming that vitamin A supplementation does not reduce the incidence of infections (4, 27). Therefore, there is a need for experimental investigations to settle this clinically important issue.

Previous experimental studies showed that vitamin A deficiency is associated with a reduced number of lymphocytes (29), low immunoglobulin (Ig) concentrations (12), decreased antibody responses to viral and bacterial antigens (14, 26), impaired T-cell responses in vivo and in vitro (10, 24), and abnormal cytokine production (8, 35). Thus, vitamin A deficiency leads to a suppression of many of the effector functions of the immune system and consequently to a state of immunodeficiency. Deficiencies in the immune system are known to increase the risk of acquiring infections such as septic arthritis (16, 23). Consequently, we have reported that vitamin A-deficient animals developed arthritis upon intestinal colonization with an *Escherichia coli* strain (33). In contrast to previous studies, in which we showed that vitamin A-deficient animals had impaired antibody responses (34, 35), these animals elicited substantial antibacterial antibody levels. However, despite the high antibody levels, the animals were systemically invaded by the bacteria. These results indicate that factors other than antibodies may be important for the protection against arthritis.

Therefore, we wanted to further investigate the development of arthritis as well as to analyze changes in innate and adaptive host immune responsiveness during the course of an acute bacteremia and subsequent chronic infection in vitamin A-deficient animals. These two aspects were studied by inoculating vitamin A-deficient rats intravenously with *Staphylococcus aureus*, one of the major human pathogens causing septicemia and septic arthritis.

MATERIALS AND METHODS

Animals and diets. Male 4-week-old inbred Wistar rats, weighing 60 g each, were obtained from BK Universal, Stockholm, Sweden. They were divided into two groups of 10 rats and weaned either onto a vitamin A-free diet (ALAB, Stockholm, Sweden) or paired fed a normal, vitamin A-sufficient diet as previously described (34). The experiment was started 4 weeks later, when the rats had reached a weight plateau, which is one of the first clinical signs of vitamin A deficiency.

Bacterial strain and administration of the bacteria. *S. aureus* AB-1, which is coagulase and catalase positive and produces staphylococcal enterotoxin A (SEA), was used for inoculation (7). The rats were given intravenous injections with 5×10^8 CFU of bacteria in phosphate-buffered saline (PBS).

Clinical evaluation. From the day of bacterial inoculation until they were sacrificed 18 days later, the rats were individually examined for their general appearance, changes in weight, and development of arthritis. The limbs were inspected visually each day by at least two observers. Arthritis was defined as swelling and/or erythema of at least one joint and was scored from 0 to 3 for each paw as previously described (7).

Sampling. Blood samples were obtained before and 24 h, 7 days, and 18 days after bacterial inoculation. Serum was stored at −20°C until analyzed for Ig, antibody, and cytokine levels. Bile was collected under pentobarbital anesthesia by inserting a plastic tube (PE 20, Intramedic; Clay Adams, Parsippany, N.J.) into the bile duct (11) and was stored at −20°C until analyzed. Liver biopsy specimens were frozen at −20°C for analysis of the retinol levels as previously described (9, 19). Spleens were obtained aseptically, and mononuclear cell suspensions were prepared. The organs were minced and filtered through sterile nylon filters (Becton Dickinson, Paramus, N.J.). The erythrocytes were lysed, and the cells were then washed and resuspended in Iscove's complete medium (10% fetal calf serum, 1% L-glutamine, 1% gentamicin) to the proper concentration.

Bacterial cultures. Bacterial samples from the arthritic and nonarthritic joints were collected with charcoal-covered sticks after dissection of the talocrural and

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subtalar joints of the hindpaw or the radiocarpal joints of the forepaw. The samples were cultured on 5% horse blood agar plates. Kidneys and livers were excised aseptically and cut with a sterile scalpel, and impression cultures from the exposed surfaces were transferred to 5% horse blood–agar plates. The bacterial samples were tested for catalase and coagulase activity.

Histopathologic examination. After sacrifice, the limbs were removed and fixed in paraformaldehyde. The processing was followed by decalcification, routine paraffin embedding, sectioning, and staining with hematoxylin and eosin.

Immunoglobulin concentrations. Levels of IgG and IgM in serum and of IgA in bile were measured by enzyme-linked immunosorbent assay (ELISA).

Affinity-purified goat anti-rat IgG (1 μg/ml in PBS; Zymed, San Francisco, Calif.), affinity-purified rabbit anti-rat IgM (1 μg/ml in PBS; Zymed), and affinity-purified goat anti-rat IgA (2 μg/ml in PBS; Saxon, Hannover, Germany) were used for coating microtiter round-bottom plates (MIC-2000; Dynatech Lab. Inc., Alexandria, Va.). The serum samples were diluted 1/2,500 and 1/500 in PBS-Tween for IgG and IgM antibody detection, respectively, and the bile samples were diluted 1/500 in PBS-Tween for IgA antibody detection. For IgG determination, alkaline phosphatase (APH)-conjugated goat anti-rat IgG (1/300; Zymed) was used. IgM and IgA concentrations were detected by a two-step ELISA with monoclonal mouse anti-rat IgM antibody and mouse anti-rat IgA antibody (1/3,000, Zymed) followed by APH-conjugated affinity-purified goat anti-mouse Ig (1/4,000; Tago, Inc., Burlingame, Calif.). The ELISA was developed with APH substrate (1 mg/ml) (no. 104 phosphatase substrate; Sigma, St. Louis, Mo.) in 1 M diethanolamine buffer (pH 9.8), and the A_{405} was measured on a spectrophotometer (Titertek Multiscan; Flow Labs, MacLean, Va.). The Ig concentrations were estimated from standard curves obtained with IgG, IgM, or IgA myeloma proteins (Pharmingen, Stockholm, Sweden) of known concentrations.

Antibody levels. Levels of antibodies to cell walls of *S. aureus* AB-1, single-stranded DNA and SEA were measured by ELISA. Microtiter plates were precoated with poly-L-lysine (25 μg/ml in PBS) and then coated with 100 μl of whole, formalin-treated (4% formalin for 20 min) *S. aureus* AB-1 cells (10^8/ml of PBS) or directly coated with 50 μg of heat-denaturated (boiled for 20 min and then cooled on ice) calf thymus DNA (Sigma) per ml or 1 μg of SEA (Sigma) per ml. Serum samples (diluted 1/100) and bile samples (diluted 1/10) were further diluted in fivefold steps. Rabbit anti-rat IgG (1/10,000; Zymed), mouse anti-rat IgM, and mouse anti-rat IgA antibodies (1/3,000; Serotec, Oxford, England) were applied, followed by APH-conjugated goat anti-rabbit Ig (1/10,000; Sigma), or APH-conjugated goat anti-mouse Ig (1/4,000; Tago). The antibody activity was expressed in arbitrary ELISA units, which were estimated from a standard curve obtained with a pool of hyperimmune sera.

Rheumatoid factors. Levels of IgG and IgM rheumatoid factors in serum were measured by a diffusion-in-gel ELISA (13) as previously described (31).

IL-6. The level of interleukin-6 (IL-6) in serum was measured with the cell line B 13.29, subclone B9, which is dependent on interleukin-6 for its growth (18). The B9 cells, seeded at a concentration of 5,000 cells per well, were incubated for 68 h with the serum samples, which were added to the wells in twofold dilutions. During the last 4 h of the culture, the cells were pulsed with [^{3}H]thymidine (Amersham, Amersham, England) and harvested (Inotech; Ninolab, Upplands Väsby, Sweden), and beta-radiation was analyzed (Matrix 96; Canberra Packard, Uppsala, Sweden). Recombinant rat IL-6 (Genzyme, Cambridge, Mass.) was used to create a standard curve (1).

Cell proliferation assay. Spleen cell suspensions were seeded into 96-well flat-bottom culture plates (Nunc, Kamstrup, Denmark) at a concentration of 4×10^5 cells per well. Concanavalin A (ConA; 2 μg per well, Sigma), SEA (1 μg per well), or cell walls from *S. aureus* (10^7 per well) were added to the cultures for 48 h. The cultures were pulsed with 1 μCi of [^{3}H]thymidine per well and harvested after 12 to 16 h, and the proliferative responses were measured in a beta counter.

Cell cultures for cytokine production. Spleen cell suspensions were seeded into 24-well plates (Nunc) at a concentration of 2×10^6 cells per ml. The cells were stimulated for 48 h with ConA (10 μg/ml), SEA (5 μg/ml), or *S. aureus* cell walls (5×10^7/ml). The supernatants were collected and used for analyses of interleukin-2 (IL-2), gamma interferon (IFN-γ), and tumor necrosis factor alpha (TNF-α).

IL-2 bioassay. Cells from the CTLL 2 cell line (American Type Culture Collection, Rockville, Md.) were cultured for 24 h at a concentration of 2×10^4 cells per well with the supernatants from mitogen- or antigen-stimulated spleen cells. During the last 4 h, the cultures were pulsed with [^{3}H]thymidine, harvested, and counted in a beta counter. The IL-2 levels were compared with a standard curve obtained with recombinant IL-2 (Genzyme) and expressed in units per milliliter (15).

IFN-γ ELISA. Monoclonal mouse anti-rat IFN-γ antibody (1/200 in PBS; Biosource International, Camarillo, Calif.) was used for coating flat-bottom microtiter plates (Nunc). Supernatants from mitogen- and antigen-stimulated spleen cells were applied undiluted, further diluted in threefold steps, and incubated for 3 h. A two-step detection system consisting of application of rabbit anti-rat IFN-γ (1/10,000 in PBS-Tween; Biosource) for 1 h followed by APH-conjugated goat anti-rabbit IgG antibodies (1/2,000 PBS-Tween; Sigma) for a further 1 h was used. The assay was developed with APH substrate and read in a spectrophotometer as described above. Recombinant IFN-γ (a kind gift from Peter van der Meide, TNO Institute of Applied Radiology and Immunology, Rijswijk, The Netherlands) was used as a standard, and the content of IFN-γ in the supernatants was expressed in units per milliliter.

TNF-α assay. The TNF-α level in supernatants from spleen cells stimulated with *S. aureus* cell walls (10^7/ml), SEA (2 μg/ml; Sigma), or ConA (10 μg/ml; Sigma) was determined by using a TNF-α ELISA kit (Genzyme).

Determination of C3. The C3 level in serum was determined by single radial immunodiffusion (22). Sheep anti-rat C3 antibodies (whole serum; Binding Site, Birmingham, England), diluted 1/100 in PBS, were incorporated into a 0.7% agarose gel containing 6% polyethylene glycol (3000 PEG). The serum samples were diluted 1/2 and incubated for 3 days at room temperature. The gels were stained with 0.1% Coomassie blue solution, the diameters of the precipitation rings were measured, and the areas were calculated. The C3 levels were expressed as a percentage of a standard derived from normal rat sera.

Complement activity test. The complement activity in the sera from *S. aureus*-infected and noninfected vitamin A-deficient and control animals was tested by hemolysis of sheep erythrocytes (SRBC). SRBC (1% in physiological saline solution) were incubated with bovine serum albumin (BSA; 50 μg/ml) for 2 h at 37°C and then washed twice. Then the solution was incubated with heat-inactivated, undiluted rabbit anti-BSA antiserum for 30 min at 37°C and washed twice. Heat-inactivated (56°C for 30 min) and non-heat-inactivated serum samples, diluted in twofold steps, were incubated with the amboceptor-coated SRBC in a 1:1 ratio for at least 2 h at 37°C. The highest serum dilution leading to hemolysis of the SRBC was used to express the complement activity of serum.

Phagocytic capacity of peripheral blood cells. The number and activity of circulating phagocytic cells were determined with a commercial kit (Orpega Pharma, Heidelberg, Germany). Freshly drawn heparinized blood from uninfected vitamin A-deficient and control rats was incubated with fluorescein isothiocyanate-labelled *S. aureus* cells (10^9/ml) for 10 min at 37°C. The samples were then transferred to ice to stop the phagocytosis. The samples were washed, membrane-bound fluorescein isothiocyanate was quenched, and the cell membranes were permeabilized. The percentage of monocytes and granulocytes which showed phagocytosis and their phagocytic activity, expressed as the mean fluorescence intensity per cell, were determined by flow cytometry (Becton-Dickinson, Mountain View, Calif.).

Phagocytosis and intracellular killing by peritoneal macrophages. The phagocytosis test was performed by a modification of a previously described method (20). In brief, peritoneal macrophages from uninfected vitamin A-deficient and control animals were obtained by injecting 20 ml of ice-cold Iscove's cell culture medium into the peritoneal cavity and aspirating the medium 1 to 2 min later after vigorous massage of the abdomen. The macrophages were adjusted to 2×10^6/ml of medium, seeded in 200-μl volumes in 24-well plates (Nunc), and incubated at room temperature for 90 min. Thereafter, 500 μl of medium was added to each well, and the cells were incubated for another 4 to 6 h at 37°C. The medium was removed, and 500 μl of medium without any antibiotics was added to the cells. After incubation overnight, the cells were washed once with Iscove's medium, and 500 μl of an *S. aureus* suspension was added at a concentration of 2×10^6 bacteria per ml for 50 min. The cells were washed three times with Iscove's medium to remove bacteria which were not ingested. Thereafter, the macrophages were analyzed at three different time intervals, i.e., directly after 50 min of incubation with the bacteria (time zero) and 4 and 24 h later. To those macrophages which were incubated for 4 and 24 h, Iscove's medium containing gentamicin (2 μg/ml) was added to avoid any extracellular replication of the bacteria. The macrophages were then lysed with destilled water for 20 min, and the lysate, diluted 1/1, 1/10, and 1/100, was cultured on 5% horse blood–agar plates. The plates were incubated for 24 h at 37°C, and the number of bacteria was counted.

Statistics. The Mann-Whitney U test and the Student *t* test were used for statistical analysis.

RESULTS

Vitamin A levels. The average retinol levels in serum, measured at the end of the experiment, were 0.3 μmol/liter (standard deviation [SD] = 0.2; $n = 4$) in the vitamin A-deficient rats and 1.6 μmol/liter (SD = 0.5; $n = 4$) in the control rats. Accordingly, the retinol levels in the liver were also significantly lower in the vitamin A-deficient rats (<5 IU/g; $n = 4$) than in the controls (1,500 IU/g; $n = 4$). The results show effective nutritional vitamin A depletion.

Clinical and histopathological evaluation. From the time of bacterial inoculation until sacrifice 18 days after the inoculation, the weight increase of the vitamin A-deficient rats was about four times lower than that of the control animals, despite equal food intake (Fig. 1). Within the first 8 days of infection, three of the vitamin A-deficient rats and one of the control rats died as a consequence of the *S. aureus* septicemia. At the end of the experiment, six (86%) of the seven vitamin A-deficient

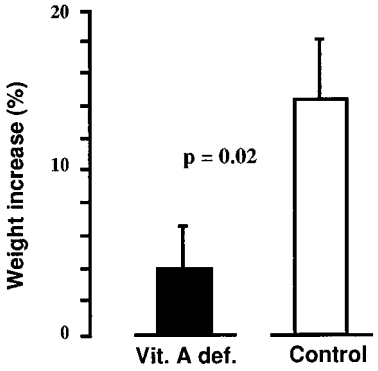

FIG. 1. Mean percent weight increase in seven vitamin A-deficient and nine control rats from day 0 until day 18 after inoculation with *S. aureus*. At the time of bacterial inoculation, the mean body weight of the vitamin A-deficient rats was 256 g (SD, 13), whereas at the end of the experiment, 18 days later, the body weight increased to 266 g (SD, 23). The corresponding data for the body weight of the controls were 200 g (SD, 7) and 229 g (SD, 23), respectively.

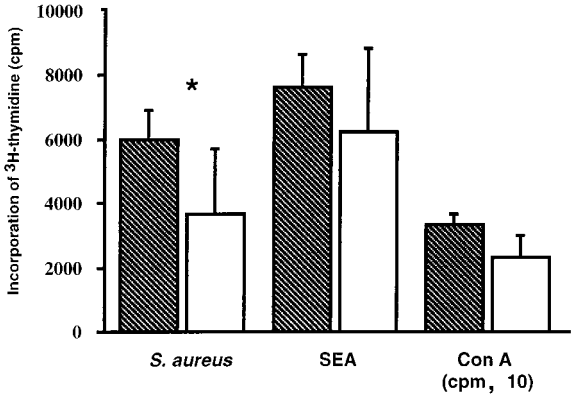

FIG. 3. T-cell responses to *S. aureus*, SEA, and ConA in spleen cell cultures from seven vitamin A-deficient and nine control rats. The bars represent the mean cpm values. *, $P < 0.05$. Symbols: ▨, vitamin A-deficient rats; ▭, controls.

surviving rats but only four (44%) of the nine control rats had developed arthritis. However, the difference did not reach statistical significance. Histopathological evaluation of the joints revealed erosive changes in 71% of the vitamin A-deficient rats and in 55% of the control rats. Interestingly, whereas most of the controls developed arthritis within the first 5 days after bacterial inoculation and then gradually recovered, the frequency of arthritis within the deficient group increased continuously throughout the experimental period (Fig. 2).

Bacteriological findings. Growth of *S. aureus* was found in the kidneys of all animals and in the livers of three rats from each group. In contrast, severe lesions such as kidney abscesses were observed in 57% of the deficient rats but only in 11% of the control rats. Furthermore, *S. aureus* was isolated from the joints of 70% of the vitamin A-deficient rats and from 40% of the control animals. The differences were, however, not statistically significant.

T-cell proliferative responses. Proliferative responses after stimulation with *S. aureus* cell walls were significantly higher ($P < 0.05$) in spleen cell cultures from the infected vitamin A-deficient rats than in those from the infected control rats. Stimulation with SEA and ConA also resulted in higher T-cell responses in the cell cultures from the deficient rats than from the control rats, but the differences did not reach statistical significance (Fig. 3). Analysis of the T-cell proliferative responses to ConA and SEA in the uninfected rats with and without vitamin A deficiency showed a similar pattern (for ConA, deficient rats [$n = 6$] had 13,987 cpm [SD = 5,574] and control rats [$n = 6$] had 6,446 cpm [SD = 2,642], $P = 0.01$; for SEA, deficient rats had 2,758 cpm [SD = 910] and control rats had 741 [SD = 355], $P = 0.0005$).

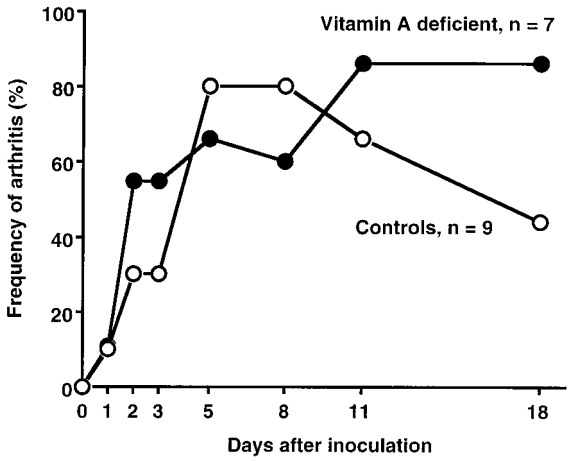

FIG. 2. Frequency of septic arthritis in seven vitamin A-deficient and nine control rats from the day of bacterial inoculation until sacrifice on day 18. The difference was not statistically significant.

Cytokine levels in serum and in vitro cytokine production. The IL-6 levels peaked with an 8- to 10-fold increase after 24 h (deficient rats, 520 pg/ml [SD = 122]; control rats, 485 pg/ml [SD = 222]) and declined slightly until day 18 (deficient rats, 380 pg/ml [SD = 193]; control rats, 261 pg/ml [SD = 145]). At all time points, the IL-6 IL-2, IFN-γ, and TNF-α levels were measured in supernatants from mitogen- and antigen-stimulated spleen cells. No significant differences in the different cytokine levels could be found between the groups, although the IFN-γ levels tended to be higher in the deficient animals (112 U/ml [SD = 60]) than in the controls (87 U/ml [SD = 70]).

Ig and antibody levels. IgG and IgM levels in serum and IgA levels in bile did not differ between the groups at the end of the experiment. Antibody levels against bacterial cell walls and SEA increased during the infection, but there were no differences between the groups. The levels of RF and anti-single-stranded DNA antibodies increased two- to fivefold from the day of bacterial inoculation in both groups.

Phagocytosis and intracellular killing capacity. The number of phagocytic cells in the peripheral blood of the vitamin A-deficient rats was 30% higher than in the control animals. However, the mean uptake of bacteria per phagocyte, expressed as fluorescence intensity, was significantly lower in the deficient rats than in the control rats (1,494 [SD = 314] versus 1,811 [SD = 219]; $P = 0.02$). These results were further confirmed by an in vitro method with peritoneal macrophages. The number of ingested bacteria being recovered from the macrophages at time zero, i.e., after 50 min of incubation with *S. aureus*, was about half as much ($P < 0.01$) in the deficient animals (Fig. 4A). Also, the intracellular killing capacity of the peritoneal macrophages from the vitamin A-deficient rats was significantly impaired. After 4 and 24 h of bacterial incubation, the number of viable *S. aureus* organisms recovered from the macrophages was around 10 times higher ($P < 0.001$) and 8 times higher ($P < 0.001$), respectively, in the deficient rats than in the control rats (Fig. 4B).

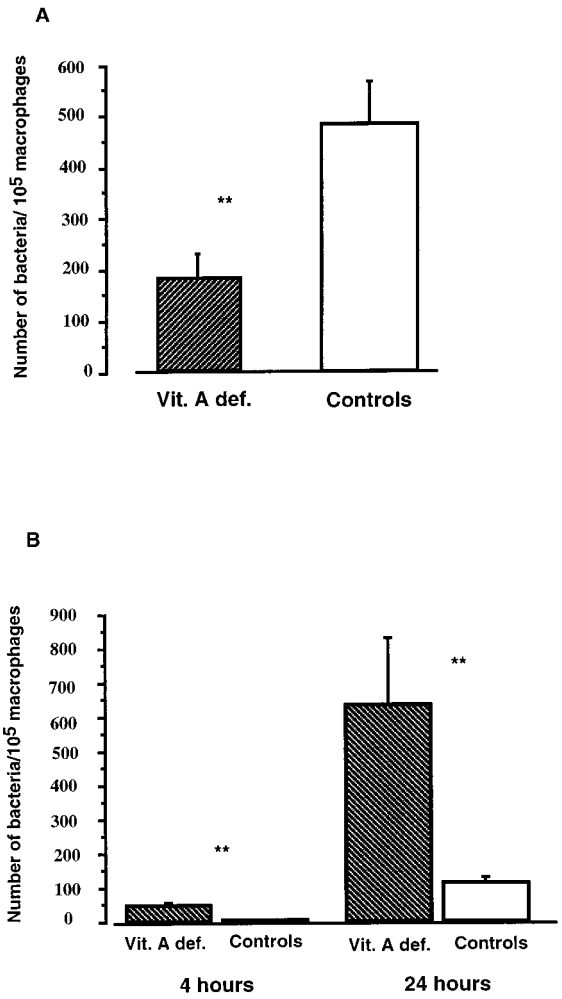

FIG. 4. (A) Phagocytic capacity. Shown is the number of viable, phagocytized *S. aureus* organisms in the peritoneal macrophages originating from vitamin A-deficient and control animals after 50 min of incubation of the cells with *S. aureus*. **, $P < 0.01$. (B) Intracellular killing capacity. Shown is the number of viable *S. aureus* organisms in the peritoneal macrophages from vitamin A-deficient and control rats 4 and 24 h after bacterial incubation. **, $P < 0.01$.

Complement activity and C3 levels. The complement activity in sera from infected and uninfected animals was measured. While the sera from uninfected vitamin A-deficient and control rats did not differ in their capacity to lyse SRBC, the sera from *S. aureus*-infected vitamin A-deficient rats had a 50% reduction in the hemolytic activity compared with the sera from the infected but vitamin A-sufficient control animals ($P = 0.04$) (Fig. 5). None of the heat-inactivated sera showed any hemolysing activity.

The levels of C3 in serum did not differ between the groups. Mean C3 levels, expressed as the percentage of a standard of normal rat sera, in sera from infected animals were 76% in the deficient rats and 75% in the control rats. The C3 levels in uninfected control rats were 83%, which was slightly higher than those in the uninfected deficient rats, which were 75% of the standard serum level.

DISCUSSION

In the present study, we showed that vitamin A deficiency led to an increased susceptibility to *S. aureus*-induced morbidity and mortality. This increase occurred although the vitamin A-deficient rats, because of a higher initial weight, received a relatively smaller bacterial inoculum than the controls did. During the experimental period, three of the deficient rats but only one of the control rats died. The controls developed arthritis within the first week, with a peak at day 5. Thereafter, the majority resolved the infection, since at the end of the experiment only four of nine control animals had arthritis. In contrast, the number of deficient rats developing arthritis gradually increased with the duration of the experiment, so that all except one rat had acquired arthritis at the end of the experiment. Not only the frequency but also the severity of arthritis, as demonstrated by histopathological signs of erosivity, was increased in the vitamin A-deficient rats. An important parameter for the clinical evaluation of the severity of an infection is change in weight. The fact that the weight increase of the vitamin A-deficient rats was approximately four times lower than that of the controls, despite equal food intake, indicated a more severe infection process leading to an increased catabolism in these animals.

What are the mechanisms whereby vitamin A deficiency increases the susceptibility to *S. aureus* arthritis? In this respect, we have previously shown that T cells (2, 7) and B cells (36) participate in the development of septic arthritis. The present study does not demonstrate any major differences between vitamin A-deficient and control animals with regard to polyclonal and antigen-specific B-cell activation. In contrast, our study shows that the T cells in the *S. aureus*-inoculated, vitamin A-deficient rats were hyperreactive. Such highly activated T cells may also be found in the arthritic joints of mice (3) and rats with *S. aureus* arthritis (7). In situ production of

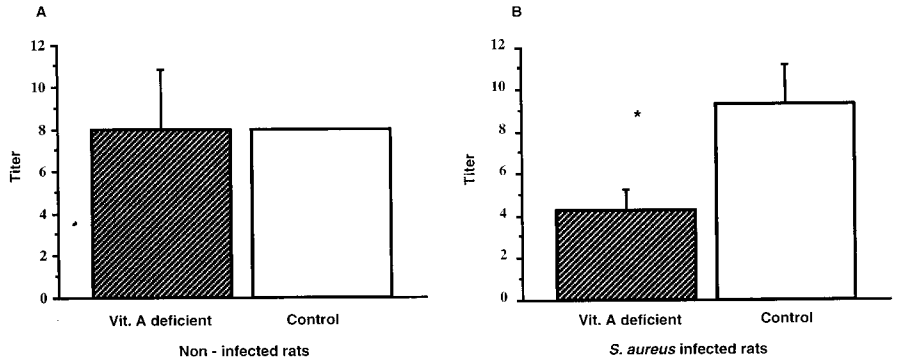

FIG. 5. (A) Complement activity, measured by the lysis of SRBC, in sera from uninfected vitamin A-deficient and control rats. (B) Complement activity in sera from *S. aureus*-infected vitamin A-deficient and control rats. *, $P < 0.05$.

proinflammatory cytokines, as demonstrated in our previous studies (3), may have a decisive role in the development of erosive joint changes. The finding of increased levels of IFN-γ in serum in the *S. aureus*-inoculated deficient rats compared with those in the control rats could be interpreted as the outcome of a more severe infection rather than the primary cause of the arthritis. However, we have previously shown that vitamin A-deficient, noninfected rats have an increased IFN-γ production (35), which may contribute directly to the severe course of the disease.

The main host defense systems against *S. aureus* infection are supposed to consist of phagocytes and the complement system (17). Our recent finding that blockade of sialophorin (CD43), expressed predominantly on phagocytic cells, aggravates the course of *S. aureus* infection (6) prompted us to assess the impact of vitamin A deficiency on the phagocytosis and intracellular killing of staphylococci. The results obtained in the present study suggest that the increased occurrence of bacteria in, e.g., the joints of the vitamin A-deficient rats might be due to phagocytic deficiencies. Thus, despite large numbers of circulating phagocytes, the ability of the cells to ingest and kill *S. aureus* was profoundly impaired in the vitamin A-deficient animals. These findings are supported by a recent study showing that vitamin A deficiency decreases the ingestion capacity of phagocytes as well as their ability to reduce nitroblue tetrazolium (28). The capacity to reduce nitroblue tetrazolium, although previously used as a measure of bactericidal activity, has recently been shown not to be directly correlated to cytotoxicity (5). Consequently, as we have demonstrated here, the capacity to kill *S. aureus* is strongly reduced whereas the ability to kill *E. coli* is less affected in the vitamin A-deficient rats (unpublished observation). Hence, vitamin A deficiency might cause an increased susceptibility to certain infections whereas the resistance to other microorganisms might be less strongly affected.

Besides the phagocytes, the complement system is important for defense against bacteria, and complement deficiencies have been shown to be a risk factor for the development of bacterial arthritis (16). Previous studies on bacterial clearance in vitamin A-deficient rats showed that the animals did not have decreased complement levels and that vitamin A deficiency did not affect the complement system (21, 25). Neither did we detect any difference with respect to C3 levels in sera from infected or uninfected vitamin A-deficient and control rats. However, we could demonstrate a reduced capacity of the complement system to lyse SRBC. Of special interest was that only the *S. aureus*-infected vitamin A-deficient rats displayed a 50% reduction of the hemolysing activity whereas no difference was found between the uninfected vitamin A-deficient and control rats. It appears that simple immunochemical measurements of complement components as performed by us and others does not permit us to draw conclusions directly as to the functional capacity of the complement cascade. Thus, anti-C3 antisera might have detected not only intact C3 but also fragments of this molecule. Alternatively, the infectious process per se in vitamin A-deficient animals may lead to decreased production and/or increased consumption of complement products downstream of C3, such as the membrane attack complex. Taken together, our results indicate that some disturbances in the host defense mechanisms during vitamin A deficiency might not be clinically apparent unless an infection, e.g., by *S. aureus*, is superimposed on the host.

In summary, we have shown that vitamin A deficiency leads to an increased susceptibility to *S. aureus*-induced arthritis and mortality. Three mechanisms have been described, which are likely to have influenced the infectious process during vitamin A deficiency. The impaired function of the phagocytes, the decreased complement activity, and the increased T-cell reactivity may all contribute to the aggravation of the infection and the increased morbidity in the vitamin A-deficient animals.

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REFERENCES

1. **Aarden, L. A., E. R. Groot, O. L. Shaap, and P. M. Landsdorp.** 1987. Production of hybridoma growth factor by human monocytes. Eur. J. Immunol. **17:**1411–1416.
2. **Abdelnour, A., T. Bremell, R. Holmdahl, and A. Tarkowski.** 1994. Clonal expansion of T lymphocytes causes arthritis and mortality in mice infected with toxic shock syndrome toxin-1-producing staphylococci. Eur. J. Immunol. **24:**1161–1166.
3. **Abdelnour, A., T. Bremell, R. Holmdahl, and A. Tarkowski.** 1994. The role of T lymphocytes in experimental *Staphylococcus aureus* arthritis. Scand. J. Immunol. **39:**403–408.
4. **Arthur, P., B. Kirkwood, D. Ross, S. Morris, J. Gyapong, A. Tomkins, and A. Hutton.** 1992. Impact of vitamin A supplementation on childhood morbidity in northern Ghana. Lancet **339:**361–362.
5. **Bellinati-Pires, R., M. Carneiro-Sampaio, and G. M. Colletto.** 1992. Functional evaluation of human neutrophils. Is the bactericidal activity correlated with nitroblue tetrazolium reduction? J. Invest. Allergol. Clin. Immunol. **2:** 146–153.
6. **Bremell, T., R. Holmdahl, and A. Tarkowski.** 1994. Protective role of sialophorin (CD43)-expressing cells in experimental *Staphylococcus aureus* infection. Infect. Immun. **62:**4637–4640.
7. **Bremell, T., S. Lange, R. Holmdahl, C. Ryden, G. Hansson, and A. Tarkowski.** 1994. Immunopathological features of rat *Staphylococcus aureus* arthritis. Infect. Immun. **62:**2334–2344.
8. **Carman, J., and C. E. Hayes.** 1991. Abnormal regulation of IFN-γ secretion in vitamin A deficiency. J. Immunol. **147:**1247–1252.
9. **Catignani, G. L., and J. G. Bieri.** 1983. Simultaneous determination of retinol and α-tocopherol in serum or plasma by liquid chromatography. Clin. Chem. **29:**708–712.
10. **Chandra, R. K., and B. Au.** 1981. Single nutrient deficiency and cell-mediated immune response. Nutr. Res. **1:**181–185.
11. **Dahlgren, U. I., S. Ahlstedt, T. Andersson, L. Hedman, and L. Å. Hanson.** 1983. IgA antibodies in rat bile are not solely derived from thoracic duct lymph. Scand. J. Immunol. **17:**569–574.
12. **Davis, C. Y., and J. L. Sell.** 1989. Immunoglobulin concentrations in serum and tissues of vitamin A-deficient broiler chicks after Newcastle disease virus vaccination. Poult. Sci. **68:**136–144.
13. **Elwing, H., and H. Nygren.** 1979. Diffusion-in-gel enzyme-linked immunosorbent assay (DIG-ELISA): a simple method for quantification of class-specific antibodies. J. Immunol. Methods **31:**101–107.
14. **Friedman, A., and D. Sklan.** 1989. Antigen-specific immune response impairment in the chick as influenced by dietary vitamin A. J. Nutr. **119:** 790–795.
15. **Gillis, S., M. M. Ferm, and K. A. Smith.** 1978. T cell growth factor: parameter of production and quantitative microassay for activity. J. Immunol. **120:** 2027–2032.
16. **Goldenberg, D., and J. Reed.** 1985. Bacterial arthritis. N. Engl. J. Med. **312:** 764–771.
17. **Goldenberg, D. L., P. L. Chisholm, and P. A. Rice.** 1983. Experimental models of bacterial arthritis: a microbiologic and histopathologic characterization of the arthritis after intraarticular injection of *Neisseria gonorrhoeae*, *Staphylococcus aureus*, group A streptococci, and *Escherichia coli*. J. Rheumatol. **10:**5–11.
18. **Helle, M., L. Boeije, and L. A. Aarden.** 1988. Functional discrimination between interleukin-6 and interleukin-1. Eur. J. Immunol. **18:**1535–1540.
19. **Hinds, F. C., A. P. Peter, and G. E. Richards.** 1968. Methods of extracting vitamin A from the liver. J. Anim. Sci. **27:**1678–1681.
20. **Lissner, C. R., R. Swanson, and A. D. O'Brien.** 1983. Genetic control of the innate resistance of mice to *Salmonella typhimurium*: expression of the Ity gene in peritoneal and splenic macrophages isolated *in vitro*. J. Immunol. **131:**3006–3013.
21. **Madjid, B., S. Sirisinha, and A. Lamb.** 1978. The effect of vitamin A deficiency and protein deficiency on complement levels in rats. Proc. Soc. Exp. Biol. Med. **158:**92–98.

22. **Mancini, G., and C. O. Carbonara.** 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry **2:**235–254.
23. **Mikhail, I., and G. Alarcon.** 1993. Nongonococcal bacterial arthritis. Rheum. Dis. Clin. North Am. **19:**311–331.
24. **Nauss, K., D. Mark, and R. Suskind.** 1978. The effect of vitamin A deficiency on the in vitro cellular immune response of rats. J. Nutr. **109:**1815–1823.
25. **Ongsakul, M., S. Sirisinha, and A. J. Lamb.** 1985. Impaired blood clearance of bacteria and phagocytic activity in vitamin A deficient rats. Proc. Soc. Exp. Biol. Med. **178:**204–208.
26. **Pasatiempo, A. M., M. Kinoshita, C. E. Taylor, and A. C. Ross.** 1990. Antibody production in vitamin A-depleted rats is impaired after immunization with bacterial polysaccharide or protein antigens. FASEB J. **4:**2518–2527.
27. **Rahmathulla, L., B. A. Underwood, R. D. Thulasiraj, and R. C. Milton.** 1991. Diarrhoea, respiratory infections and growth are not affected by a weekly low dose vitamin A supplement: a masked, controlled field trial in children in Southern India. Am. J. Clin. Nutr. **54:**568–577.
28. **Sijtsma, S. R., J. H. Rombout, M. J. Dohmen, C. E. West, and A. J. Van der Zijpp.** 1991. Effect of vitamin A deficiency on the activity of macrophages in Newcastle disease virus infected chickens. Vet. Immunol. Immunopathol. **27:**17–27.
29. **Sijtsma, S. R., J. H. Rombout, A. Kiepurski, O. West, and A. J. Zijpp.** 1991. Changes in lymphoid organs and blood lymphocytes induced by vitamin A deficiency and Newcastle disease virus infections in chicks. Dev. Comp. Immunol. **15:**349–356.
30. **Sommer, A., E. Djunaedi, A. A. Loeden, and R. Tilden.** 1986. Impact of vitamin A supplementation on childhood mortality, a randomized controlled community trial. Lancet 1169–1173.
31. **Tarkowski, A., C. Czerkinsky, and L. Å. Nilsson.** 1984. Detection of IgG rheumatoid factor secreting cells in autoimmune MRL/1 mice: a kinetic study. Clin. Exp. Immunol. **58:**7–12.
32. **West, K. P., G. R. Howard, and A. Sommer.** 1989. Vitamin A and infection: public health implications. Annu. Rev. Nutr. **9:**63–86.
33. **Wiedermann, U., L. Å. Hanson, T. Bremell, H. Kahu, and U. I. Dahlgren.** 1995. Increased translocation of *Escherichia coli* and development of arthritis in vitamin A-deficient rats. Infect. Immun. **63:**3062–3068.
34. **Wiedermann, U., L. Å. Hanson, J. Holmgren, H. Kahu, and U. I. Dahlgren.** 1993. Impaired mucosal antibody response to cholera toxin in vitamin A-deficient rats immunized with oral cholera vaccine. Infect. Immun. **61:**3952–3957.
35. **Wiedermann, U., L. Å. Hanson, H. Kahu, and U. I. Dahlgren.** 1993. Aberrant T cell function *in vitro* and impaired T cell dependent antibody responses *in vivo* in vitamin A deficient rats. Immunology **80:**581–586.
36. **Zhao, Y., A. Abdelnour, R. Holmdahl, and A. Tarkowski.** 1995. Mice with the Xid defect are less susceptible to developing Staphylococcus aureus induced arthritis. J. Immunol. **155:**2067–2076.

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