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Natural killing and antibody-dependent cellular cytotoxicity are independent immune functions in the Minnesota miniature swine

(51Cr release/specific pathogen-free pigs/germfree pigs/newborn piglets/erythrocyte rosettes)

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ABSTRACT **Peripheral blood lymphocytes from Minnesota miniature pigs were tested for natural killing (NK) and antibody-dependent cellular cytotoxicity (ADCC) in a 2- to 4-hr ^{51}Cr release assay against human myeloid and lymphoid tumor target cells. Adult specific pathogen-free and germfree animals exhibited normal levels of activity in both assays. In addition, the NK and ADCC activities of peripheral blood lymphocytes from colostrum-deprived newborn piglets were examined. These animals were obtained by hysterectomy and previously shown to be immunologically "virgin." We found that these newborn piglets exhibited normal ADCC but lacked NK activity. The differences in the ontogeny of the two activities suggest that they are distinct. Preliminary effector cell characterization studies suggest that: (*i*) NK and ADCC in the pig are physically not separable; (*ii*) the majority of the cytotoxic activity on a cell-per-cell basis is mediated by the non-T lymphocyte fraction; and (*iii*) the rosetted T cells, which account for about 60% of the total pig peripheral blood lymphocytes, have low but demonstrable cytotoxic activity as well.**

Natural killing (NK), defined as the cytolytic activity of normal unsensitized lymphoid cells against (various) target cells, has been described in rodents and man [for review, see Herberman and Holden (1)]. The mechanism and specificity of NK are still unclear. It has been suggested that NK plays a part in immune surveillance against tumors (2). The ontogeny of NK and the impact of ubiquitous antigens on its development have not been thoroughly studied yet.

NK activity is detectable at birth in human cord blood lymphocytes (3); in mice, it becomes demonstrable only at approximately 3 weeks of age (4). Environmental influences and sensitization of the fetus by maternal antigens may trigger responsiveness. However, current environmental models have not been able to distinguish clearly between antigen-driven activation and antigen-independent maturation of NK effector cells (5, 6). Some investigators have suggested that NK is antibody-dependent, thus implying that NK and antibody-dependent cellular cytotoxicity (ADCC) are identical functions (7, 8).

To help answer some of these questions we have introduced two approaches: examination of the activities of lymphocytes from patients with primary immunodeficiency diseases (9) and the Minnesota miniature pig model.

The Minnesota miniature swine has long been shown to be a unique and useful model system to study ontogeny of and environmental impact on the immune response (10, 11). The placenta of pigs consists of six tissue layers, which prevent transfer of maternal immunoglobulin (Ig) and antigenic material if the placental barrier is intact (12). Moreover, one can obtain miniature piglets by aseptic hysterectomy 3–5 days before term and maintain them germfree (GF). The colostrum-deprived piglets have been shown to be immunologically "virgin," with no detectable levels of Ig but fully capable of producing antibodies 48 hr after a single injection of antigen (10, 11).

The isolation of the piglets from maternal Ig and maternally acquired antigens, the controlled environment in which they can be raised, and the access to large numbers of cells can make the Minnesota miniature pig a most attractive model to study the development of NK. We describe the establishment of sensitive short-term ^{51}Cr release assays that allowed us to study NK and ADCC activities in the pig. Both conventional and GF pigs aged 2 months or more had NK and ADCC activity in their peripheral blood. Most of the cytotoxic activity was mediated by non-T lymphocytes. However, newborn piglets, whether colostrum-deprived or fed, had ADCC activity but no detectable NK activity. These findings suggest that NK and ADCC activities are different. Furthermore, they indicate that the development of NK activity is not due to environmental immunization or maternal antibodies.

MATERIALS AND METHODS

Animals. GF colostrum-deprived piglets were obtained by aseptic hysterectomy 3–5 days prior to term from disease-free Minnesota miniature sows (10, 11). The piglets were maintained on animal protein-free soybean derivative Mullsoy diet (Syntex Laboratories, Palo Alto, CA) for long-term experiments in the GF isolators. The specific pathogen-free (SPF) miniature swine were maintained in a barrier-sustained SPF swine facility.

Mononuclear Cell Isolation. Mononuclear cells were separated from peripheral blood by the Ficoll/Hypaque density centrifugation method of Böyum (13). The lymphoid cells, which sedimented to the interface between the blood and the Ficoll/Hypaque solution, were carefully pipetted out and washed twice with balanced salt solution and resuspended in the culture medium.

Cell Transportation and Culture. Mononuclear cells used in most of the experiments described in this paper were prepared aseptically (Rye, NY). They were then shipped in containers with ice by air to Durham, NC, where they arrived on the same day. Upon arrival the cells were immediately adjusted to 2×10^6 cells per ml in RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 1 mM pyruvate, 1× nonessential amino acids, 100 units of penicillin per ml, and 100 μg of streptomycin per ml (Flow Laboratories, Rockville, MD). This medium will be referred to as "culture medium." For convenience, cells were then cultured overnight in plastic disposable 12 × 75 mm tubes (Falcon, no. 2052, Oxnard, CA) in 2- to 4-ml aliquots at $2–4 \times 10^6$ cells per ml at 37° in a

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Abbreviations: PBL, peripheral blood lymphocytes; NK, natural killing; ADCC, antibody-dependent cellular cytotoxicity; SPF, specific pathogen-free; GF, germfree; SRBC, sheep erythrocytes; E-RFC, erythrocyte rosette-forming cells; TNP, 2,4,6-trinitrophenyl.

humidified 7% CO_2 incubator. This incubation usually resulted in higher cytotoxic activities.

Removal of Adherent Cells. Cells were adjusted to 2×10^6 cells per ml in culture medium, dispensed in 20–30 ml into 75-cm^2 plastic flasks (Corning, no. 25116-75), and incubated for 1 hr at 37°. Nonadherent cells were collected and were found to contain less than 1% phagocytic cells [by latex particle ingestion (14)]. This procedure was performed on the day the cells arrived or on the following day, prior to assay.

T Lymphocyte Fractionation. T lymphocytes were fractionated from non-T cells on the basis of their ability to form rosettes with sheep erythrocytes (SRBC), which has been shown elsewhere (15) to be a reliable marker for porcine T cells. The T cell fraction was tested without removal of the SRBC unless otherwise stated. Erythrocyte rosette-forming cells (E-RFC) were enumerated directly after separation by taking an aliquot from the pellet and counting the cells under the microscope. In some of the experiments, samples of both fractions were rerosetted the next day to ascertain the relative purity by counting the number of E-RFC. The number of E-RFC in PBL of adult pigs, measured by the above technique, was about 70%.

Tumor Target Cell Lines. SB is a human B cell line (16) and K562 is a human myeloid leukemia cell line (17). The cells were maintained in stationary suspension culture in 75-cm^2 plastic flasks in the culture medium described above. The tumor cells were passed every other day, and on the day preceding an assay.

Chromium-51 Labeling of Target Cells. Between 10 and 20×10^6 cells were resuspended in 0.2 ml of minimal essential medium supplemented with 10% fetal calf serum (assay medium). $Na_2{}^{51}CrO_4$ (100 μCi, specific activity 250–500 mCi/mg of ^{51}Cr, New England Nuclear, no. NEZ-030) at a concentration of 100 μCi/50 μl was added to the target cells, which were then incubated in a 37°C water bath for 1 hr with occasional shaking. Labeled target cells were washed 3 times with assay medium and adjusted to the desired concentration.

Assay for NK. Effector cells were tested against ^{51}Cr-labeled K562 target cells in a total volume of 0.2 ml of assay medium. Triplicate determinations were made in round-bottomed microtiter plates (Linbro, Is-MRC-96TC). Effector:target (E:T) ratios varied from 100:1 to 12.5:1 with 1×10^4 targets per well. The plates were centrifuged 3 min at $80 \times g$ to facilitate cell contact, followed by a 2½ hr incubation (unless otherwise stated) at 37° in a humidified CO_2 incubator. The assay was terminated by spinning the plates for 5 min at $500 \times g$. Samples (0.1 ml) of the supernatants were collected and their radioactivities were measured in a well-type gamma counter (Searle model 1185).

Cell line K562 was used as a target for human NK assays due to its sensitivity to NK lysis (1).

Assay for ADCC. ^{51}Cr-Labeled SB cells were modified with 2,4,6-trinitrobenzene sulfonate as previously described (18); this trinitrophenyl-conjugated target cell will be referred to as "SB-TNP." This cell line has been shown to be resistant to human NK and is therefore a convenient target for ADCC assays (9). SB-TNP targets were incubated in the test wells in the presence or absence of mouse anti-TNP antiserum (final dilution of 1:3000) for 30 min at 37°C prior to the addition of effector cells. In this case, 0.05 ml of the targets, 0.05 ml of antiserum, and 0.1 ml of effectors were used. Incubation of pig (newborn and adult) effector cells with SB-TNP in the absence of anti-TNP serum in most cases did not result in a marked increase of cytolysis compared to medium control. Therefore, values for controls without antiserum are not shown, though they were determined in all the experiments.

Calculation of Cytotoxic Activity. For both the NK and ADCC assays, spontaneous release (SR) is defined as the cpm released from targets incubated in medium alone. Maximal release (MR) was determined by measuring CPM in the supernatants after lysis of the various targets with the detergent Triton X-100. The formula used to calculate the % specified release was:

$$\% \text{ specific release} = \frac{\text{cpm experimental} - \text{cpm SR}}{\text{cpm MR} - \text{cpm SR}} \times 100$$

Data were calculated and statistically analyzed by using a cytotoxicity program according to the above formula with a PDP 11/10 computer. The mean SRs of K562 and SB-TNP were 6.5 ± 3% and 11.0 ± 4%, respectively. All uncertainties are expressed as ± 1 SEM.

RESULTS

NK and ADCC Activities in Adult SPF and GF Pigs. It was necessary to establish that NK and ADCC activities could be demonstrated in the pig. Because there are no cell lines from miniature pigs available as targets, we employed xenogeneic targets, which are used to study NK and ADCC in humans (9). The human myeloid leukemia line K562 was lysed by unsensitized PBL from adult SPF and GF pigs as shown in Table 1. As with human effector cells, SB-TNP targets in the absence of anti-TNP antibodies were less sensitive to lysis (data not shown), but SB-TNP was effectively lysed by effector cells from SPF and GF pigs in ADCC in the presence of the appropriate

Table 1. NK and ADCC activities in peripheral blood mononuclear cells of SPF and GF pigs

Effector cells*				% specific lysis					
				ADCC (SB-TNP)			NK (K562)		
Exp.	Pig	Sex	Age, mo	50:1	25:1	12.5:1	50:1	25:1	12.5:1
1†	3095-3	♀	5	25.8 ± 1.9	14.6 ± 1.3	8.3 ± 1.6	6.3 ± 0.4	5.1 ± 0.9	4.1 ± 0.5
	309A-5	♀	5	50.8 ± 1.3	21.5 ± 1.1	25.8 ± 1.9	59.0 ± 0.8	45.0 ± 0.2	29.6 ± 1.1
	3006-1	♂	42	67.6 ± 1.3	43.0 ± 1.3	40.0 ± 1.3	NT	82.3 ± 0.4	47.8 ± 2.5
2‡	GF3106-4	♀	2	41.1 ± 1.5	24.8 ± 1.7	NT	64.2 ± 0.6	44.4 ± 2.1	NT
	GF3101-2	♀	3	9.2 ± 1.3	5.8 ± 0.8	NT	10.5 ± 0.8	8.0 ± 0.3	NT
	GF3091-3	♀	5	20.5 ± 0.9	19.9 ± 1.3	NT	49.9 ± 0.9	43.0 ± 1.7	NT
	GF309-4	♀	5	55.6 ± 1.0	39.7 ± 1.1	NT	74.6 ± 1.5	66.1 ± 5.2	NT
	GF3091-5	♀	5	71.7 ± 1.6	54.9 ± 1.3	NT	81.1 ± 1.5	83.1 ± 0.9	NT
	GF3080-4	♂	8	10.4 ± 0.8	8.9 ± 1.5	NT	33.8 ± 1.7	27.0 ± 0.7	NT

NT, not tested.
* PBL were incubated for 18 hr at 37° and then assayed.
† PBL of SPF pigs.
‡ PBL of GF pigs.

Table 2. NK and ADCC activities in adult pig peripheral blood mononuclear cell fractions

Effector cells*			% specific lysis					
			ADCC (SB-TNP)			NK (K562)		
Exp.	Pig	Fraction tested	50:1	25:1	12.5:1	50:1	25:1	12.5:1
1	ExGF-3091-3	Unfractionated	22.4 ± 0.6	12.4 ± 0.5	NT	27.4 ± 0.3	16.0 ± 1.2	NT
		T	0.3 ± 0.1	−0.2 ± 0.1	NT	2.1 ± 0.1	2.2 ± 0.2	NT
		Non-T	NT	30.3 ± 0.6	18.3 ± 1.0	NT	47.5 ± 1.9	39.9 ± 2.9
	ExGF-3094-4	Unfractionated	27.9 ± 1.1	18.8 ± 1.2	NT	32.4 ± 2.1	20.1 ± 1.0	NT
		T	5.0 ± 0.5	3.0 ± 0.2	NT	6.7 ± 0.2	4.7 ± 0.3	NT
		Non-T	46.9 ± 0.5	33.8 ± 0.9	NT	49.8 ± 1.1	38.0 ± 0.8	NT
2	ExGF-3094-4	Unfractionated	12.0 ± 0.1	6.0 ± 0.1	NT	13.4 ± 1.1	8.0 ± 0.1	NT
		T	3.6 ± 0.3	0.4 ± 0.1	1.2 ± 0.1	3.4 ± 0.1	2.6 ± 0.3	1.6 ± 0.5
		Non-T	16.8 ± 1.1	7.8 ± 0.9	5.2 ± 0.3	14.2 ± 0.5	8.6 ± 1.0	3.8 ± 0.9
3	3018-3	Unfractionated	10.0 ± 1.0	6.3 ± 1.0	3.5 ± 0.8	6.0 ± 0.1	3.4 ± 0.0	2.1 ± 0.0
		T	15.7 ± 0.8	14.2 ± 0.3	8.6 ± 0.9	38.7 ± 1.2	34.5 ± 1.2	18.6 ± 0.1
		T†	19.5 ± 1.2	14.8 ± 0.4	8.9 ± 0.2	39.9 ± 2.3	29.5 ± 2.0	15.4 ± 0.1
		Non-T	31.7 ± 0.3	23.5 ± 0.5	24.5 ± 0.8	57.6 ± 3.9	52.2 ± 2.5	46.2 ± 2.2
	3104-6	Unfractionated	20.2 ± 0.5	8.7 ± 0.7	4.5 ± 1.0	7.8 ± 0.7	4.6 ± 0.6	2.8 ± 0.0
		T	6.6 ± 1.1	7.5 ± 0.9	5.4 ± 0.3	12.7 ± 0.1	10.3 ± 0.1	11.0 ± 0.1
		T†	5.8 ± 1.2	5.9 ± 1.0	3.5 ± 0.3	10.1 ± 0.1	8.4 ± 0.2	4.9 ± 0.2
		Non-T	15.8 ± 0.9	10.3 ± 1.0	9.1 ± 0.9	24.8 ± 2.0	15.1 ± 0.3	11.8 ± 1.2

NT, not tested.
* PBL of adult pigs incubated for 18 hr at 37° were fractionated into T and non-T cell fractions as described in *Materials and Methods.*
† In these cell fractions the SRBC were removed by hypotonic shock.

anti-TNP antibodies. Because there is day-to-day variability in cytotoxicity, it would be misleading to draw general conclusions from the data presented in Table 1 with respect to the effect of age or sex on either NK or ADCC. The data do, however, illustrate the fact that both GF pigs, which are minimally exposed to environmental antigens, and SPF pigs exhibited significant NK and ADCC activities in a dose-dependent fashion in a short-term ^{51}Cr release assay.

Cytotoxic Activities of Fractionated Effector Cells. Because all experiments were carried out after monocyte depletion, it was reasonable to assume that monocytes were not contributing significantly to either NK or ADCC. To determine the relative contribution of both T and non-T lymphocytes, PBL were separated on the basis of the ability of T cells to form rosettes with SRBC. The rerosetting data of the fractionated cells with SRBC showed that the T cell fraction was 86 ± 10% pure and the non-T fraction contained about 10% T cells. The data presented in Table 2 demonstrate that in all the experiments the non-T cell fraction was the most active one in both assays, though significant activity was exhibited by the T cell fractions. T cell killing was especially strong in experiment 3 with cells from both of the pigs. In experiments 1 and 3 the NK and ADCC activities of the non-T cells were higher than those of unfractionated cells. In experiment 3 the T cell fraction of pig 3018-3 also showed higher reactivity than the unfractionated cells of the same pig. The presence of SRBC in the T cell fractions did not seem to interfere with either NK or ADCC activities because T cell fractions in which the SRBC were hypotonically lysed showed essentially the same activity as fractions that were not so treated (Table 2, experiment 3).

Table 3. NK and ADCC activities in peripheral blood mononuclear cells of colostrum-deprived piglets obtained by hysterectomy

Effector cells*			% specific lysis			
			ADCC (SB-TNP)		NK (K562)	
Exp.	Donor	Hours in culture prior to assay	100:1	50:1	100:1	50:1
1	Mother 3070-1	0	23.9 ± 2.9	13.1 ± 0.7	1.8 ± 0.3	3.0 ± 0.6
	Piglet 3070-1 Po-1	0	32.7 ± 1.0	NT	0.4 ± 0.3	NT
2	Mother 3070-1	18	31.0 ± 3.0	11.2 ± 0.5	7.0 ± 0.6	5.2 ± 0.5
	Piglet 3070-1 Po-1	18	46.9 ± 0.5	NT	0.1 ± 0.4	NT
3†	Mother 3062-5	18	18.0 ± 0.1	2.0 ± 4.5	15.8 ± 1.6	11.6 ± 0.1
	Piglet 3062-5 Po-1	18	NT	NT	0.0 ± 0.6	NT
	Piglet 3062-5 Po-3	18	19.0 ± 2.4	NT	0.0 ± 0.4	NT
4	Mother 3056-4	18	33.0 ± 0.6	27.4 ± 3.2	15.3 ± 2.1	7.9 ± 0.6
	Piglet 3056-4 Po-2	18	24.7 ± 2.9	21.2 ± 2.0	3.3 ± 0.2	3.2 ± 0.1
5	Mother 3037-5	18	52.5 ± 2.5	36.3 ± 7.6	36.0 ± 1.2	21.3 ± 1.0
	Piglet 3037-5 Po-1	18	28.5 ± 0.3	NT	0.5 ± 0.4	NT

NT, not tested.
* PBL obtained from the piglets on the day of hysterectomy were tested in NK and ADCC immediately (as in exp. 1, 0 hr) or incubated overnight first.
† In exp. 3 the NK assay was for 4 hr.

Cytolytic Activities of Newborn Piglets. Having determined that adult SPF and GF (but not antigen-free) pigs had NK and ADCC activities (Table 1), we investigated to what extent these functions were influenced by age. Experiments were carried out on newborn piglets obtained by aseptic hysterectomy 3 days prior to full term and shown not to have any Ig (10, 11). The experiments listed in Table 3 clearly show that PBL of the newborns had demonstrable ADCC but totally lacked NK against K562 even when tested at 100:1. NK activity was not detectable when the cells were tested on the day of hysterectomy (experiment 1) or after 18 hr incubation at 37°C (experiment 2). In some experiments (data not shown), feeding the piglets with colostrum for 2 days [which is sufficient to achieve maternal levels of Ig in the piglet (19)] still did not produce detectable NK activity. The fact that 2-month-old GF pigs did show NK activity (Table 1) suggests that maturation plays a role in the development of NK, but does not rule out antigenic stimulation as an alternative mechanism.

DISCUSSION

NK has so far been described in mice, rats, and humans (1). The possibility that NK has a role in immune surveillance against tumors (2) and in resistance to hemopoietic transplants (18) has created an increased interest in this function. In spite of extensive research, several questions related to NK are still controversial or unanswered. For example, little is known about the factors controlling the ontogeny of NK, such as the influence of environmental antigens upon its development. Similarly, the relationship between NK and K cells (effector cells in ADCC) and the role of antibodies in NK are yet unclear.

The Minnesota miniature swine has several unique features that make it an ideal system in which to study some of these problems. The most striking feature of the pig system is the fact that immunoglobulin cannot cross the placenta if the placenta barrier is intact (12). Hence, newborn piglets obtained by hysterectomy are immunologically "virgin" but fully immunocompetent (10, 11). Moreover, the technology and methodology necessary to raise the pigs under GF conditions are available (10, 11).

Our first experiments established NK and ADCC assays using pig effector cells (Table 1). Because pig cell lines were not available to us, human cell lines previously shown to be suitable targets for these assays have been employed. The human myeloid leukemia line K562 (9) is a sensitive target for human NK, whereas SB, a human B cell line (16), is relatively insensitive to NK in short-term assays but is effectively lysed in ADCC when coated with appropriate antibodies (9). With effector cells obtained from adult SPF and GF pigs the same pattern of reactivity against these target cells was observed. The assays were short (2–4 hr) at E:T ratios of 100:1 and less. Variability between individual pigs exists, though it is yet unknown to what extent factors such as age, sex, pregnancy, and genetic background are responsible for this variability.

Preliminary characterization of pig effector cells ruled out macrophages as significant contributors to either NK (K562 targets) or ADCC (SB targets) (data not shown). This finding is in accordance with many published reports on both human and rodent effector cells (1). We chose to use rosette fractionation of pig T cells with SRBC. This marker has previously been shown to be characteristic of pig T cells (15), with the frequency of T cells in pig PBL being similar to that in human PBL. After conventional rosetting techniques with pig PBL, enriched NK and ADCC activities were found in the non-T cell fractions, though significant cytolytic activities were found in the rosetted T cell fraction as well (see Table 2). The results obtained in this study suggest that the non-T cell fraction (which we assume contains B cells and Fc receptor-positive non-B cells) is the most active one on a per cell basis. These findings are in accordance with our results in humans (20) as well as with those obtained by several other authors (21–24). Further characterization of the effector cells by using other techniques based on Fc receptors and surface Ig should be informative.

In general, PBL provided a convenient and reproducible source of effector cells from adult pigs. NK and ADCC activities were also found in the PBL of GF pigs 2 months of age and older. The level of activities found in these GF animals were in the same range as those found in SPF pigs, suggesting that environmental antigens, to which these piglets are minimally exposed, are not the only factors in the development of NK activity. Thus far, our data are consistent with previous reports that did not find an important role for environmental factors in the levels of NK activity in rodents. For example, Nunn *et al.* (5) and Shellam and Hogg (6) found similar levels of NK activity in GF and conventional rats.

Experiments conducted with PBL of GF colostrum-deprived newborn piglets obtained by hysterectomy demonstrated significant ADCC activity but total lack of NK even when tested at high E:T ratios (100:1) and in longer than usual assays (4 hr) (see experiments 1 and 2, Table 3). Moreover, feeding the newborn piglets with colostrum (rich in all Ig classes), which results in maternal levels of Ig in the circulation as well as cell bound within a few hours (19), did not affect the early development of NK activity (data not shown), suggesting that lack of NK expression in the newborn is antibody independent. Studies of ontogeny of ADCC in mice detected high levels of activity in neonates (25, 26). In studies of NK ontogeny, activity was present from the second to third week of life but was absent at birth (4). These studies in the newborn mice seem to agree with our findings in the piglet, even though the Ig levels and neonatal exposure to maternal antigens of the two species at birth are totally different. These findings support the hypothesis that NK and ADCC activities are mediated by different mechanisms, as suggested in other model systems (27, 28). We have already shown a dichotomy between the two activities in X-linked agammaglobulinemia patients (who lack surface-Ig^+ B cells and circulating Ig). Their PBL lacked effector cells for ADCC (29) but had demonstrable levels of NK (9). The development of NK in piglets under environmentally controlled conditions requires further study.

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1. Herberman, R. B. & Holden, H. T. (1978) in *Advances in Cancer Research*, eds. Klein, G. & Weinhouse, S. (Academic, New York), Vol. 27, pp. 305–377.
2. Haller, O., Hanson, M., Kiessling, R. & Wigzell, H. (1977) *Nature (London)* **270**, 609–611.
3. Baines, M. G., Pross, H. F. & Millar, K. G. (1978) *Am. J. Obstet. Gynecol.* **130**, 741–744.
4. Kiessling, R., Klein, E., Pross, H. & Wigzell, H. (1975) *Eur. J. Immunol.* **5**, 117–121.
5. Nunn, M. E., Djeu, J. Y., Glaser, M., Lavrin, D. H. & Herberman, R. B. (1976) *J. Natl. Cancer Inst.* **56**, 393–399.
6. Shellam, G. R. & Hogg, N. (1977) *Int. J. Cancer* **19**, 212–224.
7. Akira, D. & Takasugi, M. (1977) *Int. J. Cancer* **19**, 747–755.
8. Pape, R. G., Troye, M. & Perlmann, P. (1977) *J. Immunol.* **118**, 1925–1930.
9. Koren, H. S., Amos, D. B. & Buckley, R. H. (1978) *J. Immunol.* **120**, 796–799.

10. Kim, Y. B., Bradley, S. G. & Watson, D. W. (1966) *J. Immunol.* **97,** 52–63.
11. Kim, Y. B. (1975) in *Immunodeficiency Diseases in Man and Animals,* Birth Defects: Original Articles Series, No. 1, eds. Bergsma, D., Good, R. A. & Finnstad, J. (Sinauer, Sunderland, MA), Vol. 11, pp. 549–557.
12. Setcavage, T. M. & Kim, Y. B. (1976) *Infect. Immun.* **13,** 600–607.
13. Böyum, A. (1968) *Scand. J. Clin. Lab. Invest. Suppl. 97* **21,** 77–89.
14. Winchester, R. J. & Ross, G. (1976) in *Manual of Clinical Immunology,* eds. Ross, N. R. & Friedman, H. (American Society for Microbiology, Washington, DC), pp. 64–76.
15. Kim, Y. B. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33,** 766.
16. Koide, Y. & Takasugi, M. (1976) *J. Immunol.* **117,** 1197–1202.
17. Lozzio, C. B. & Lozzio, B. B. (1973) *J. Natl. Cancer Inst.* **50,** 535–538.
18. Kiessling, R., Hochman, P. S., Haller, O., Shearer, G. M., Wigzell, H. & Cudkowicz, G. (1977) *Eur. J. Immunol.* **7,** 655–663.
19. Kim, Y. B. & Watson, D. W. (1966) *Ann. N. Y. Acad. Sci.* **133,** 727–745.
20. Kall, M. A. & Koren, H. S. (1978) *Cell. Immunol.* **40,** in press.
21. Jondal, M. & Pross, H. (1975) *Int. J. Cancer* **15,** 596–605.
22. Kiuchi, M. & Takasugi, M. (1976) *J. Natl. Cancer Inst.* **56,** 575–582.
23. Bakacs, T., Gergely, P. & Klein, E. (1977) *Cell. Immunol.* **32,** 317–328.
24. Kiessling, R., Klein, E. & Wigzell, H. (1975) *Eur. J. Immunol.* **5,** 112–117.
25. Ralph, P., Nakoinz, I. & Cohn, M. (1973) *Nature (London) New Biol.* **245,** 157–158.
26. Pollack, S. B. & Kraft, D. S. (1977) *Cell. Immunol.* **34,** 1–9.
27. Kay, H. D., Bonnard, G. D., West, W. H. & Herberman, R. B. (1977) *J. Immunol.* **118,** 2058–2065.
28. Nelson, D. L., Bundy, B. M. & Strober, E. (1977) *J. Immunol.* **119,** 1401–1405.
29. Sanal, S. W. & Buckley, R. H. (1978) *J. Clin. Invest.* **61,** 1–10.