across all castes and communities. Screening of healthy population is required to determine the carrier rates and gene frequencies in this region.


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Enhanced activation of mouse NK cells by IL2 in the presence of circulating immune complexes

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Circulating immune complexes (CICs) generated during a variety of diseased conditions, comprise aggregates of immunoglobulin molecules and trapped antigens. Aggregated antibodies in CICs present multiple cross-linked Fc regions of antibodies which can potentially bind low-affinity Fc receptors (FcRs) expressed on a variety of leukocyte populations. Natural killer (NK) cells constitute a population of lymphocytes with an important role in natural immune responses. NK cells express low-affinity FcRs (CD16 receptors) and can therefore be potentially activated by CICs. If CICs can influence NK cell activation, they may play an important immune-regulatory role in clinical conditions associated with elevated blood levels of CICs. In the present study, the effect of mouse-serum-derived CICs was studied on interleukin-2-induced NK cytotoxicity. In mouse bone marrow cells cultured in vitro. CICs induced a marked dose-dependent increase in NK activation response. While addition of normal monomeric IgG preparation had no effect on NK activation, aggregates of pure mouse IgG molecules prepared by mild heat treatment had an effect similar and comparable to that of CICs. These results suggest that the boosting of NK activation response by CICs could be mediated by aggregated immunoglobulin molecules present in CICs. We suggest that CICs generated in a variety of diseased conditions may act as immunomodulators for NK cells.

A wide variety of Fc receptors (FcRs) with high or low affinities for the Fc portion of immunoglobulins, have been described. Mast cells express very high affinity receptors (FcεRI) for IgE that remain saturated with monomeric IgE molecules in situ. Cross-linking of IgE molecules fixed on the mast cell surface by allergens triggers the release of bioactive mediators responsible for immediate hypersensitivity reaction. Most other leukocytes express FcRs, but these receptors have lower binding affinities and do not bind monomeric immunoglobulin molecules. Low-affinity FcRs on leukocytes can nonetheless bind multiple immunoglobulin molecules immobilized on a surface, e.g. antibody-coated pathogens, as a result of increased avidity of binding. In neutrophils and macrophages, low-affinity IgG receptors play a crucial role in recognition and phagocytosis of opsonized (antibody-coa-

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interact with CICs. Natural killer (NK) cells express FcRs, which may interact with CICs. Many studies in the literature have examined the relationship between blood levels of CICs and cytotoxic activity associated with NK cells derived from peripheral blood. These reports suggest that CIC levels may have a positive correlation with NK cell cytotoxicity. A clear verdict on the effect of CICs on NK cell cytotoxicity is therefore not yet available. Moreover, a possible influence of CICs on the NK activation process has not been explored. In the present communication, we have studied the effect of CICs on interleukin-2 (IL2)-induced activation of NK cells. Our results indicate that IL2-induced NK activation is significantly better in the presence of CICs. Implications of these results have been discussed.

Inbred C57Bl/6 mice (8 to 12-weeks-old) were used throughout the study. All the animals were bred and maintained in the animal house facility in Jawaharlal Nehru University (JNU), New Delhi. All experimental protocols were approved by JNU Institutional Animal Ethics Committee. Until otherwise specified, all the culture work was done in RPMI-1640 from Sigma, supplemented with 10% foetal calf serum (FCS), 2 × 10^{-5} M 2-mercaptoethanol (ME), 300 µg/ml glutamine and 60 µg/ml gentamycin (complete medium). Isolation of bone marrow cells and the in vitro protocol for activation by IL2 have been described earlier. Briefly, mouse bone-marrow cells were cultured at 5 × 10^6/ml with 50 U/ml of human recombinant IL-2 in the complete medium. After two days, the cultures were split into two and supplemented with equal volume of fresh medium and IL-2. On the fourth day the activated cells were washed and used as effector cells in a 4-h chromium release assay of cytotoxicity.

Circulating immune complexes were prepared by the procedure described earlier. Briefly, serum samples were diluted twofold with 0.2 M EDTA solution (pH 8.4) and CICs precipitated by adding drop-wise 12% (w/v) PEG solution (approx. molecular weight 8000, from Sigma) in borate buffer (0.1 M, pH 8.4). The mixture was incubated overnight at 4°C followed by centrifugation at 1700 g for 20 min at 10°C. CICs in pellet were washed twice in washing buffer (2.5 g PEG, 0.58 g NaCl, 0.95 g sodium tetra borate, 2.98 g EDTA and 0.12 g tris were dissolved in 90 ml distilled water, pH 7.4 adjusted with 1 N NaOH and the volume made up to 100 ml) and dissolved in phosphate-buffered saline. Heat-aggregated IgG preparations were made from commercially available purified mouse IgG preparations, as described earlier. Chromium release assay was performed using YAC lymphoma tumour target cells, as described earlier. The per cent lysis values at different effector/target ratios were converted into lytic units/10^6 effector cells using a computer program developed by David Coggin, National Institutes of Health, Bethesda, MD.

NK cells express low-affinity FcRs, which may interact with CICs. Fresh mouse bone-marrow cells are devoid of NK1.1+ cells, but after four days of culture with IL2 the concentration of NK1.1+ cells ranges from 20 to 25% (Figure 1). Results in Figure 1 show that about 55% of the NK1.1+ cells expressed CD16 (low-affinity FcR for IgG). The aim of the present study was to assess if CICs can influence the activation of NK cells. For this purpose, bone-marrow cells were cultured for four days with IL2 with or without various doses of CICs and anti-YAC cytotoxic activity of the activated preparations was assessed at several effector/target ratios. BM cells cultured with IL2 had significant cytotoxic activity, which could be further boosted by CICs (Figure 2). Effect of CICs was dose-dependent and among the doses tested, maximum effect was observed at 50 µg/ml dose of CICs (Figure 2). Cytotoxic activity of bone marrow cells cultured in the absence of IL2 has not been shown because few bone marrow cells survived in this condition.
CICs comprise aggregates of immunoglobulin molecules and trapped antigen. Fc portions of complexed IgG molecules in CICs may interact with CD16 receptors on NK cells and trigger an activating signal responsible for the observed boosting response. Results in Figure 3 show that the monomeric IgG preparation did not boost NK activation, indicating that low-affinity interaction between monomeric IgG molecules and the FcγRs on NK cells may not be sufficient to generate the activation signal. Since CICs have components other than IgG molecules, it could be argued that non-Ig components of CICs were responsible for boosting NK activation response. If this proposition is correct, aggregates of pure IgG should not boost the NK activation response. Aggregates of IgG can be generated by a mild heat treatment and heat-aggregated IgG preparations are known to effectively bind FcγRs. Heat-aggregated IgG preparations had a dose-dependent NK boosting effect comparable to that of CICs (Figure 4). Taken together, our results suggest that CICs may boost NK cell activation and this effect is likely to be mediated by aggregated immunoglobulins present in CICs.

These results may have practical implications. While CIC levels are very low in healthy individuals, there is a considerable increase in CIC levels in a variety of diseased conditions. Blood levels of CICs in various diseased conditions range from 40 µg/ml and above. Our results show that CICs could boost NK activation response within this very dose range. CIC levels rise significantly in cancer patients and a CIC-induced NK activation response in these patients may be beneficial. Effects of CICs on the activities of neutrophils, T- and B-lymphocytes, and platelets have also been demonstrated. Immune res-
Response and its regulation are processes that require a complex interplay of a variety of agents, including cytokines. It is tempting to speculate that CICs generated during various types of immune responses may also participate in the immunoregulatory network by signalling through FcRs expressed on different classes of leukocytes.

17. Bayer, A. S., Theofilopoulos, A. N., Tillman, D. B., Dixon, F. J. and Guze, L. B., Use of circulating immune complex levels in the serodifferentiation of endocarditic and nonendocarditic septice-